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HYPERCALCEMIC SYNDROME ASSOCIATED WITH ANDROGENIC AND ESTROGENIC THERAPY

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IN RECENT years androgenic and estrogenic compounds have been employed in the treatment of breast carcinoma (1-7). Previous work has suggested that these hormones may occasionally cause hypercalcemia in patients with osteolytic metastatic mammary carcinoma (6, 8). Spontaneous hypercalcemia occurs not infrequently in debilitated patients suffering from osteolytic lesions secondary to mammary carcinoma. We have encountered six such instances in the past year and in some of these patients there have been recurrent episodes of hypercalcemia associated with nausea and vomiting.¹ Since hypercalcemia is the only serious complication reported to date apparently attributable to sex hormone therapy it appeared to be important to establish, if possible, the relationship of these hormones to the production of hypercalcemia.

The case histories are reported of four patients, three of whom developed hypercalcemia coincident with the administration of testosterone propionate and one with the administration of diethylstilbestrol. Three patients had mammary carcinoma and one patient had a reticulum-cell sarcoma. They were in the terminal stage of the disease and received hormonal therapy after all other therapeutic measures had been employed.

Case 1. (38687R) E.W., a 38-year-old white woman underwent a right radical mastec-

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¹ Report to be published.

tomy about five and one-half years prior to her admission to the Montefiore Hospital in February 1947. Three years after the mastectomy she developed pleural, pulmonary, osseous and pelvic metastases for which she was surgically castrated. Subsequent to the castration she received high voltage irradiation to the metastatic areas. Some palliation was obtained from these therapeutic procedures. Later the patient's condition

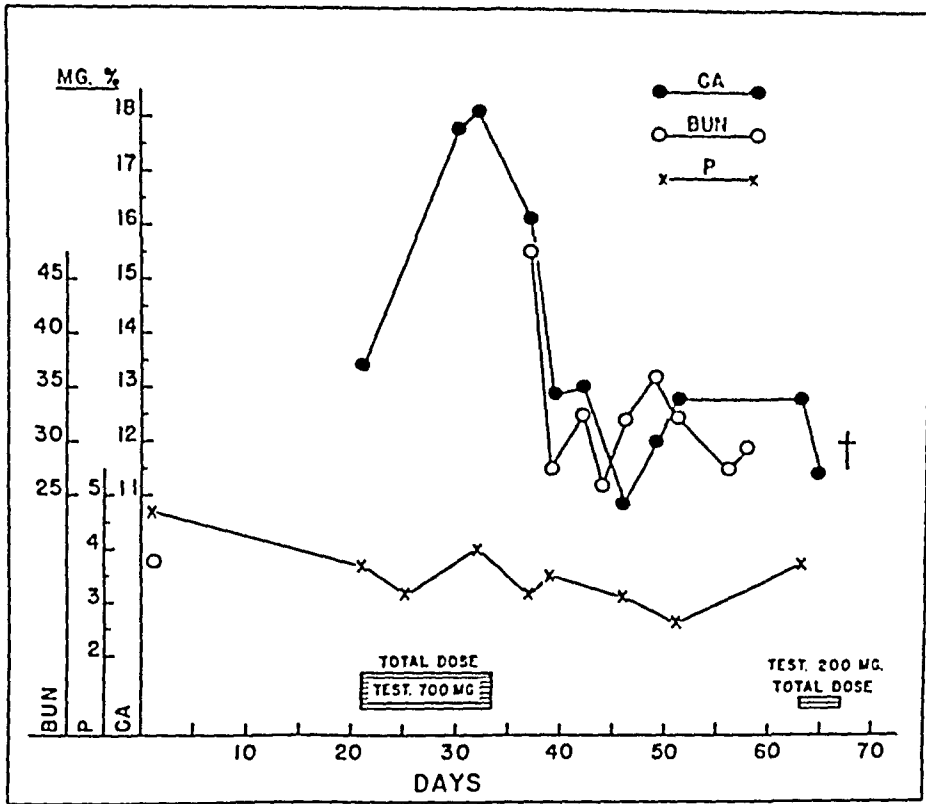


FIG. 1. Testosterone propionate administered to a patient with hypercalcemia caused a rapid elevation of the serum calcium. After termination of the initial period of testosterone therapy the serum calcium and blood urea nitrogen levels fell but did not return to normal levels. One month later a small amount of the hormone was administered for a period of four days. The patient died subsequently (time of death indicated by dagger).

deteriorated and she was admitted to the hospital because of generalized weakness and pain in the right hip.

Physical examination on admission to the hospital revealed numerous nodules in the skin of the right chest wall in the region of the mastectomy scar that were interpreted as metastatic mammary carcinoma. Roentgenologic studies disclosed evidence of widespread osteolytic and pulmonary metastasis. Aspiration of the sternum revealed almost complete replacement of the marrow by neoplastic cells. The blood urea nitrogen (BUN) and serum phosphorus were within normal limits.

Testosterone therapy was initiated three weeks after admission. The patient received 700 mg. of testosterone propionate over a period of twelve days. The serum calcium, which was somewhat elevated (13.5 mg. per cent) at the beginning of the treatment rose

to 18.1 mg. per cent on the eleventh day. The BUN was 47.3 mg. per cent five days later (Fig. 1). The urea clearance at this time was 33 per cent of normal.

Nausea and vomiting developed several days after the androgen therapy was initiated. The vomiting increased in severity and the patient later became lethargic. Her condition improved after the therapy was discontinued and large amounts of glucose, saline and 2.5 per cent sodium citrate, the latter in 250 cc. quantities, were administered parenterally. Concomitant with this improvement there was a decrease in the serum calcium and BUN values. The urinary output, which at the height of toxicity was 200 to 300 cc. daily, increased to over 2000 cc. daily. During the month subsequent to the withdrawal of testosterone the serum calcium and BUN remained moderately elevated and the urea clearance low. There were no toxic symptoms. The possibility existed that the hypercalcemia and azotemia were spontaneous and that their association with the administration of testosterone was coincidental.

About one month after termination of the first course of androgen therapy, testosterone propionate was again administered to the patient because of intractable pain. She received 50 mg. a day for four days. Therapy was terminated because she developed nausea and vomiting. The patient later became lethargic. Vigorous intravenous therapy was instituted but she became comatose and died four days after withdrawal of the testosterone. The serum calcium did not rise during the second and fatal toxic episode.

Postmortem examination revealed a recurrence of anaplastic mammary adenocarcinoma in the operative scar with metastasis to the left breast, lungs, thyroid, adrenals, left ureter, intestinal serosa, vertebrae, right innominate bone, sternum and ribs. Tumor obstruction of the left ureter had caused a hydronephrosis with compensatory hypertrophy of the right kidney.

Histopathologic examination of the right kidney disclosed narrowing of the lumina of the convoluted tubules with marked swelling and vacuolization of the tubular epithelium. Many calcium deposits were present in the renal tubules. There was metastatic calcification in the left lung and gastric mucosa. The left kidney was hydronephrotic. The parathyroids were normal grossly and microscopically.

Case 2. (MH42786) J.Y., a 48-year-old white woman had undergone a left radical mastectomy for carcinoma followed by roentgen castration two and one-half years prior to her admission to the Montefiore Hospital in April 1947. Her chief complaint on admission was generalized skeletal pain which had necessitated complete bed rest for a number of weeks. Physical examination revealed no evidence of recurrent disease but roentgenologic studies disclosed evidence of extensive osteolytic metastases.

The patient had received 900 mg. of testosterone propionate during the six days prior to admission and 300 mg. was administered during the first three hospital days. A serum calcium determination at this time was 13.6 mg. per cent; therefore testosterone therapy was stopped. Two days later the serum calcium was 14.6 mg. per cent and then it slowly returned to normal limits (Fig. 2). At no time was there any nausea, vomiting or lethargy.

Testosterone therapy was again instituted ten days after the serum calcium level had returned to normal limits, the patient receiving 50 mg. of the hormone every other day over a period of three weeks for a total dose of 650 mg. There was a gradual rise in the serum calcium from 9.6 mg. per cent to a peak of 16.1 mg. per cent reached six days after the therapy was terminated. There was a concomitant rise in the BUN. The urea clearance at this time was 22 per cent of normal. The hormone was withdrawn because the patient developed nausea, vomiting and lethargy. Glucose in saline and sodium

citrate solutions were administered parenterally and there was a gradual return to normal levels of the serum calcium and BUN and a concomitant cessation of the nausea and vomiting.

After a period of three weeks during which several determinations of the serum calcium and BUN revealed normal levels, testosterone therapy was once again instituted for intractable pain, the patient receiving 350 mg. of the hormone during a twelve-day period. The serum calcium quickly rose from a level of 9.1 mg. per cent to a peak of 17.2 mg. per cent three days after the withdrawal of the hormone. There was a concomitant rise in the BUN. Nausea and vomiting developed in association with the hypercalcemia.

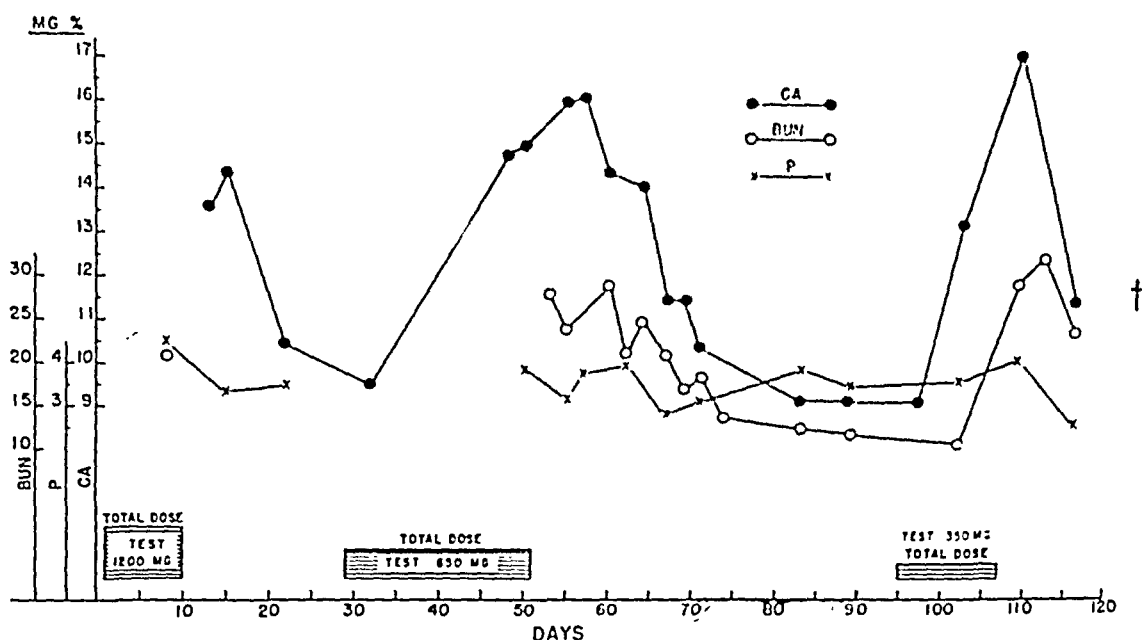


FIG. 2. Each succeeding episode of hypercalcemia was more intense, appeared more promptly and was produced by a smaller dose of testosterone propionate. Despite the fall in serum calcium and blood urea nitrogen levels associated with corrective therapy the patient died in uremic coma seventeen days after withdrawal of the testosterone propionate (time of death indicated by dagger).

Despite withdrawal of the hormone and the institution of vigorous parenteral glucose and saline therapy which was followed by a drop in the serum calcium and BUN, the vomiting persisted, the patient became lethargic, developed oliguria and died in uremic coma seventeen days after termination of the hormonal therapy. There was a terminal hyperpyrexia of 108° F.

Postmortem examination, limited to an abdominal incision, revealed metastatic mammary carcinoma in the lungs, pleura, tracheobronchial lymph nodes, liver and bones. The parathyroids were not obtained for examination.

Histopathologic examination of the kidneys revealed small areas of subcapsular fibrosis with round cell infiltration and an occasional hyalinized and fibrosed glomerulus. The glomeruli for the most part were normal. The tubules revealed degeneration of the lining epithelium and contained hyaline and granular casts. Calcium deposits were present, especially in the lumina of the collecting tubules, frequently involving the walls.

Case 3. (42410) M.R., a 40-year-old cachectic white woman had become aware of pain in both hips, fatigue and weight loss about one year prior to her admission to the Montefiore Hospital in April 1947. Five months subsequent to the onset of the pain in her hips she noted a painless mass in the left infraclavicular region and shortly thereafter similar masses in both supraclavicular areas. Biopsy of the infraclavicular mass revealed reticulum-cell sarcoma. Two months later intra-abdominal masses were discovered for

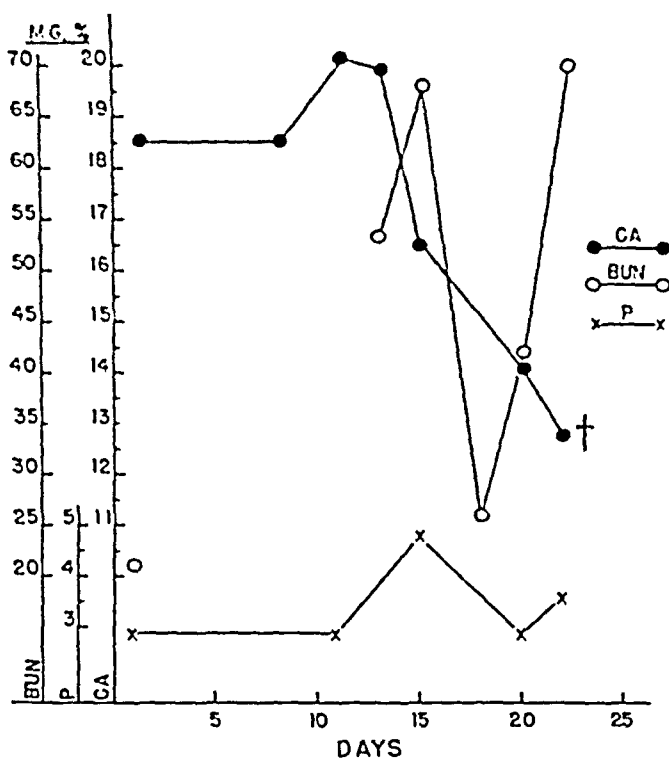


FIG. 3. The patient had received 1115 mg. of testosterone propionate for one month prior to admission to the hospital. No androgen therapy was administered after her admission. Following vigorous parenteral fluid administration there was a fall in the serum calcium and blood urea nitrogen levels. The low nitrogen level however was not sustained but rose until the patient's death (indicated by dagger).

which the patient received irradiation. Dyspnea and cough developed during this period.

The patient had been given 1115 mg. of testosterone propionate by her local physician during the month prior to her admission. Listlessness and nausea associated with frequent attacks of vomiting had been present during this month. No testosterone was administered during her hospital stay.

Physical examination on admission disclosed a nontender cervical, supraclavicular and inguinal adenopathy, bilateral pleural effusion, hepatosplenomegaly and nontender masses in the abdomen and pelvis. Roentgenologic studies revealed a moderate demineralization of all the vertebrae with collapse and anterior gibbus formation of the

eighth dorsal and erosion of the body of the fourth lumbar vertebrae. The patient was semiambulatory.

The serum calcium on admission was 18.5 mg. per cent, the BUN 21.4 mg. per cent and the phosphorus 2.8 mg. per cent (Fig. 3). The serum calcium rose to a maximum level of 20.1 mg. per cent on the eleventh hospital day and the BUN reached a peak of 68.8 mg. per cent on the fifteenth day at which time the serum phosphorus was 4.8 mg. per cent and the urea clearance 18 per cent of normal. Concomitant with these changes there were persistent vomiting, lethargy and oliguria. Following intensive therapy with parenteral glucose in saline the serum calcium fell gradually to a level of 12.6 mg. per cent on her twenty-second hospital day. During this period there was also a temporary drop in the blood urea nitrogen and phosphorus, a moderate improvement in urinary output and some decrease in the toxic symptoms. Renal insufficiency with a rise in BUN supervened, however, and the patient died on her twenty-third hospital day.

Postmortem examination revealed reticulum-cell sarcoma of the spleen, liver, pancreas, right adrenal gland, urinary bladder, ureters, uterus, cervix, vagina and vertebrae. There was congestion and edema of the lungs and marked dilatation of the stomach. The parathyroids grossly and microscopically were not remarkable.

Histopathologic examination revealed the kidneys to be congested, the capsules irregularly thickened and covered with a hemorrhagic fibrinous exudate. There was pronounced swelling of the renal tubules with numerous areas of vacuolar and hyaline droplet degeneration but no glomerular damage. Many of the larger collecting tubules contained red blood cells and the tubules of the cortex and medulla contained calcium deposits.

Case 4. (42708) D.S., a 58-year-old cachectic white woman had undergone a modified right radical mastectomy about five years prior to her admission to the Montefiore Hospital in March 1947. Her chief complaints on admission were pain in the lower back and dyspnea. The patient was semiambulatory. Roentgenologic studies disclosed massive pulmonary and multiple osteolytic metastases.

Estrogenic hormone in some instances has caused regression of pulmonary metastases secondary to breast cancer in elderly women (1, 2, 3, 4, 5). On this basis, diethylstilbestrol therapy, 2 mg. administered intramuscularly three times a day, was instituted on May 5, 1947. Several days later the dose was increased to 5 mg. three times a day until a total of 78 mg. had been administered over a period of fourteen days. Shortly after therapy was instituted the patient began to complain of anorexia and weakness. This was interpreted as an immediate reaction which occurs frequently in the diverse conditions for which diethylstilbestrol is employed. However, when listlessness and depression developed the estrogen therapy was terminated. Intensive treatment with parenteral fluids produced a gradual symptomatic improvement.

The chemical changes in the blood coincident with the toxic manifestations are shown in Fig. 4. Unfortunately the pretreatment chemical studies are incomplete. A hypercalcemia of 16.4 mg. per cent was found two days after institution of estrogen therapy. A peak of 19.9 mg. per cent was reached five days later and then the level fell precipitously during the remainder of the period of administration of diethylstilbestrol. The serum BUN and phosphorus followed a similar pattern. A urea clearance determination during this period was 25 per cent of normal. Clinical improvement became noticeable after withdrawal of the drug and institution of parenteral fluid therapy. Subsequently the patient's general condition remained satisfactory and the serum calcium, phosphorus and BUN remained within normal limits.

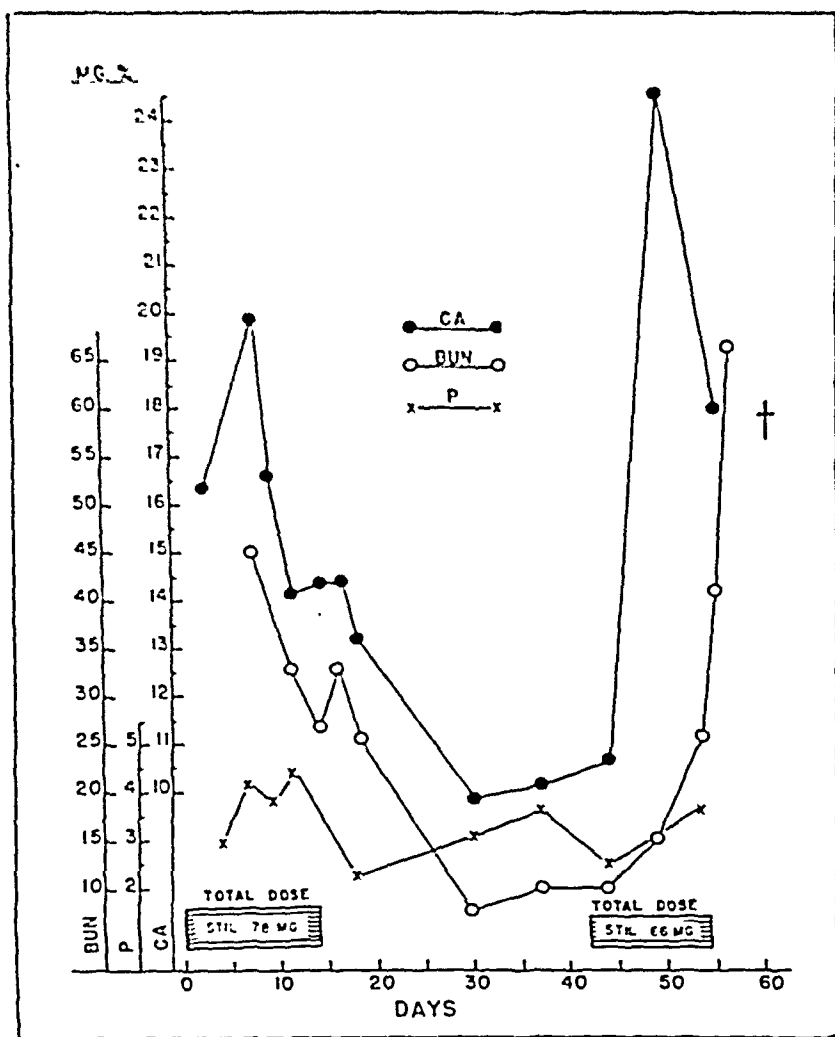


FIG. 4. There was an elevation of the serum calcium and blood urea nitrogen levels associated with diethylstilbestrol administration. After reaching a peak these levels fell during the continued administration of the estrogen. A subsequent episode with greatly elevated calcium and blood urea nitrogen levels was produced by a smaller amount of the estrogen and resulted fatally (indicated by dagger). The calcium level, as in the first episode, fell during the course of the estrogen therapy.

After the levels of calcium, phosphorus and BUN in the patient's serum had been within normal limits for several weeks diethylstilbestrol therapy was again instituted because of persistent pain and dyspnea. A total of 66 mg. was administered over a period of twelve days. The urea clearance was 29 per cent of normal prior to the second course of stilbestrol therapy. The serum calcium rose from 10.2 mg. per cent to a peak of 24.5 mg. per cent within seven days and then decreased during the continued administration of the estrogen. The clinical toxic manifestations were similar to those previously described.

Vigorous parenteral glucose and saline therapy was instituted but the patient died in coma five days after withdrawal of the estrogen.

Postmortem examination revealed anaplastic mammary adenocarcinoma metastatic to the lungs, pleura, thyroid, pituitary, thymic area, sternum, ribs, liver, left adrenal, periesophageal, perigastric, periaortic and mesenteric lymph nodes, the dorsolumbar vertebrae and the right ilium.

Histopathologic examination of the kidneys revealed dilatation of the tubules with vacuolization and granular degeneration of the tubular epithelium. Numerous areas of calcific deposits were found in the tubular epithelium (Fig. 5). Nests of tumor cells were



FIG. 5. The deeply stained areas are calcium deposits in the renal tubules.

seen in the peripelvic lymphatics. The thyroid contained many foci of tumor cells and numerous deposits of calcium were scattered throughout the gland. The parathyroid glands were moderately hyperplastic and composed of small chief cells and occasional oxyphilic cells.

DISCUSSION

Steroid hormones apparently exert a profound influence on calcium metabolism. Irradiated ergosterol mobilizes calcium and may produce hypercalcemia in dogs and in man (9, 10). Estrogens and androgens may decrease the urinary and fecal excretion of calcium and convert a negative into a

positive calcium balance in patients with osteoporosis (11). Androgenic hormones may produce in some women roentgenologic evidence of calcification in areas of osteolysis secondary to breast carcinoma (6, 7).

Occasionally toxemia, associated with hypercalcemia and a subsequent azotemia, follows the administration of androgens and estrogens to women with osteolytic lesions. Four examples of this phenomenon are reported here. In three patients there were osteolytic lesions secondary to mammary cancer and in the fourth the osteolytic lesions were associated with reticulum-cell sarcoma. It would appear that the property possessed by these hormones to produce hypercalcemia is not specific for cases of osteolytic mammary cancer. The production of hypercalcemia in patients with osteolytic mammary carcinoma following the administration of natural estrogens has been reported (8); but this is the first time to our knowledge that this phenomenon has been produced by a synthetic estrogen.

The hypercalcemia induced by the sex hormones caused nausea, vomiting and dehydration which precipitated the renal toxemia. The excretion through the kidneys of calcium mobilized by the sex hormones produced a calcinosis of the renal tubules with progressive renal impairment. Ultimately the kidneys were so badly damaged that the excretion of calcium and nitrogenous waste products was greatly diminished with resultant renal insufficiency. A similar phenomenon has been described in connection with the administration of parathyroid hormone (12) and with ergosterol therapy (9, 13, 14). It is quite possible that the degree of renal impairment present before the institution of hormonal therapy was a factor in determining the rapidity of onset of this toxic phenomenon.

It is significant that the patient (Case 1) who succumbed to a relatively small amount of testosterone was found at autopsy to have one hydro-nephrotic kidney and calcinosis of the opposite one. Reinstitution of hormonal therapy with smaller amounts of the sex hormones in Cases 1, 2 and 4 produced a more prompt and severe toxic reaction than was encountered in the first episode. In Case 1 the second course of testosterone proved fatal without producing an elevation of the serum calcium. The increase in toxicity of the hormones noted in successive periods of hormonal therapy was apparently the result of cumulative renal damage.

In addition to pre-existent renal damage a contributory factor in the production of the hypercalcemia may be immobilization. Hypercalcemia, calcinuria and renal lithiasis may occur in individuals who are immobilized even for relatively short periods of time (15). Mobilization of calcium with the production of osteoporosis is also associated with cachexia. The patients described here who developed hypercalcemia after sex hormone therapy exhibited varying degrees of cachexia and were either bedridden

or semiambulatory. We have not encountered sex hormone-induced hypercalcemia in ambulatory patients.

It may be possible to realize the therapeutic possibilities of the sex hormones in the treatment of cancer and to reduce the incidence of hypercalcemic toxemia as a complication of this therapy by the judicious selection of patients. Patients with pretreatment hypercalcemia preferably should not receive sex hormone therapy. Those who are bedridden or whose renal function is markedly impaired should be treated cautiously. Frequent blood chemical and kidney function studies should be performed. Adequate fluid intake should be maintained at all times. Lethargy, nausea, vomiting and an increase in serum calcium or evidence of progressive renal impairment should be a warning to abandon sex hormone therapy.

In addition to the discontinuation of hormonal therapy there is one measure which may be employed for the purpose of reducing the amount of ionized calcium (Ca^{++}) in the blood and tiding the patients over the critical period. This consists in the administration of sodium citrate in a 2.5 per cent solution in amounts of 250 cc. at four-hour intervals until symptomatic improvement is apparent (16). This measure achieves its effect by converting the relatively insoluble Ca^{++} ions into soluble, weakly ionized calcium citrate ions. This conversion will not alter the total serum calcium values as determined by the conventional analytical methods but will alter the ratio between the Ca^{++} and (Ca citrate^-) ions. Its administration should be discontinued once the serum calcium values have fallen to the normal levels. Overdosage with sodium citrate can be avoided by watching for the development of a Chvostek reaction, indicative of impending tetany.

The intravenous infusion of glucose, saline and of sodium citrate after withdrawal of the androgen was followed in Cases 1, 2 and 3 by the return to lower values of the serum calcium and BUN and alleviation of toxic symptoms. In the case of the patient receiving diethylstilbestrol the serum calcium level fell and continued to fall during the period of estrogen administration although it did not return to normal levels during this period. Clinical improvement of the toxic manifestations became evident only after the withdrawal of the estrogen and the institution of parenteral fluid therapy.

SUMMARY AND CONCLUSIONS

1. In the course of therapy with testosterone propionate and diethylstilbestrol, four patients, three with osteolytic lesions secondary to mammary carcinoma and one with an osteolytic lesion of a reticulum-cell sarcoma, developed toxic reactions, associated with hypercalcemia and signs and symptoms of renal insufficiency.

2. Several of these toxic episodes were corrected by the cessation of hormonal therapy and the institution of parenteral therapy consisting of infusions of saline, glucose and sodium citrate. Subsequent toxic episodes following reinstitution of hormonal therapy proved irreversible and had a lethal outcome.

3. All the patients sustained renal damage with nephrocalcinosis.

4. The rationale is presented for the therapeutic use of sodium citrate (2.5 per cent) infusions for hypercalcemic states such as encountered in this study.

5. The hypercalcemic syndrome appears to occur relatively infrequently in patients with osteolytic lesions treated with these hormonal agents. Its incidence might be reduced by careful screening of the patients selected for hormonal therapy. Several criteria for the selection of appropriate cases are suggested.

Acknowledgments

The authors are indebted to Dr. H. M. Zimmerman and staff for the pathologic studies and to Dr. Ephraim Shorr for his suggestions and advice during this investigation. The testosterone propionate (Oreton) was kindly supplied by Dr. Edward Henderson of the Schering Corporation, Bloomfield, New Jersey.

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RENAL EXCRETION AND TUBULAR REABSORPTION OF SALT IN CUSHING'S SYNDROME AFTER INTRAVENOUS ADMINISTRATION OF HYPERTONIC SODIUM CHLORIDE*

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OF THE two mechanisms by which the kidneys regulate the excretion of salt,¹ glomerular filtration and tubular reabsorption, changes in the former have been shown to be of importance in the pathogenesis of edema observed in certain patients with congestive heart failure. In such patients, Merrill (1) has shown that markedly reduced glomerular filtration contributes to the abnormal retention of injected sodium chloride observed by Futcher and Schroeder (2). Despite the fact that the rate of glomerular filtration is frequently reduced also in patients with Addison's disease (3), such patients lose salt in the urine to an abnormally great degree. On the basis of this and other evidence, it seems likely that deficiency of adrenal hormones results in a diminished capacity of the renal tubular cells to reabsorb salt. The reverse effect (increased capacity) has not been demonstrated to occur in that form of adrenal *hyper*function which results in Cushing's syndrome. Indeed, Soffer *et al.* (4) have observed that when desoxycorticosterone acetate (DCA) and salt are administered to patients with Cushing's syndrome, these subjects excrete salt to a *greater* degree than do normal subjects similarly treated.

This investigation was undertaken in order to study tubular reabsorption of salt in patients with Cushing's syndrome. Observations have been made on the influence of an intravenous injection of a large amount of sodium chloride upon the rate of salt excretion, the renal filtration rate and plasma flow in these subjects.

METHODS

All experiments were conducted according to the following standard routine: The subjects ingested no food or fluid during the 3-hour period of

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‡ Present address: Johns Hopkins Hospital, Baltimore, Md.

¹ In the exposition which follows, we have used the term "salt" synonymously with the term "sodium chloride."

observation and no food after 6 p.m., the night before. All subjects except J. K. (control) were hospitalized. All except J. K. and L. P. had ingested for at least three days a normal diet supplying approximately 3 to 5 Gm. of sodium chloride daily (calculated from content of sodium) and were allowed additional sodium chloride from a shaker as desired. J. K. ingested a normal, salted diet not prepared in the hospital. L. P. ingested for four days preceding the experiment a normal, measured diet supplying 10 Gm. of sodium chloride daily. On the morning of the test, the glomerular filtration rate and the renal plasma flow were determined during two successive control clearance periods of about 15 minutes each by measuring the mannitol and the para-aminohippurate (PAH) clearances, respectively. Solution of sodium chloride (0.85 per cent) injected intravenously at a rate of 4 cc. per minute was the vehicle for the mannitol and PAH infusion. Immediately after the second clearance period had been terminated, 400 cc. of 5 per cent sodium chloride in water was injected intravenously in about 50 minutes. This amount of salt was sufficient to cause an average temporary increase in the plasma chloride concentration of about 13 mEq. per liter. Mannitol and PAH concentrations in the hypertonic salt solution were adjusted so as to maintain constant plasma levels of these substances. Measurements of renal clearance were made during the two approximately 25-minute periods required for the infusion of 5 per cent sodium chloride. After the hypertonic salt solution had been administered, the clearance infusion mixture used for the two preliminary periods was re-instituted and observations made for two or more 15-minute clearance periods.

Urine was collected from a catheter inserted into the bladder. Twenty cc. of sterile water followed by 20 cc. air were used to wash the bladder at the end of each collection period. Blood samples were drawn from an antecubital vein approximately at the midpoints of the clearance periods; plasma levels of mannitol, PAH, and chloride at the midpoints were obtained by interpolation between observed values.

Mannitol was determined according to the method of Smith *et al.* (5). Cadmium sulfate was used to precipitate protein from plasma and urine, as recommended by Goldring and Chasis (6). Among factors which were considered in performing the mannitol, plasma and urine blanks were non-fermentable reducing substances contributed by the plasma and urine (Factor 1); reducing substances contributed by the yeast suspension² (Factor 2); and adsorption or destruction of mannitol by the yeast suspension (Factor 3). In performing the plasma and urine blanks in the second experiment on F. B., and in the experiment on L. P., all factors were

² Starch-free yeast was donated by Anheuser-Busch Company, Inc., Saint Louis, Missouri.

compensated for. No urine blank was employed in the other experiments, and only Factor 1 was compensated for in performing the plasma blank. Details of the method employed for calculation of the mannitol blanks will be presented in a separate report (7). PAH was measured as recommended by Smith *et al.* (8). The chloride content of plasma was determined as described by Van Slyke (9); that of urine was measured by the Volhard-Arnold technique (10). Urine and plasma sodium concentrations were measured by the technique of Butler and Tuthill (11). Values for clearance rates were calculated according to the method of Möller *et al.* (12). All values for clearance rates and excretion rates for chloride and water were corrected to a standard body surface area of 1.73 square meters, calculated from the DuBois height-weight formula (13).

SUBJECTS

The experimental subjects included 3 patients with Cushing's syndrome and 3 other subjects who served as controls. None of the control subjects manifested arterial hypertension or clinical evidence of renal disease. Two patients with Cushing's syndrome (G. S. and F. B.) had hypertension. Case summaries relating to the patients with Cushing's syndrome are presented below.

G. S., Cushing's syndrome (Barnes Hosp. 143750): This 35-year-old white woman was admitted February 6, 1947, because of abdominal swelling, weight gain, reddening of the skin, hypertension, and glycosuria, developing over a 3-year period following a hysterectomy for bleeding. Examination revealed a moon-shaped face, obese neck and shoulders, thin extremities and skin, arteriolar narrowing in the optic fundi, normal visual fields, rales at both lung bases, an enlarged heart, distended abdomen, violet striae over the abdomen and breasts, and a blood pressure averaging 170/110. Blood counts were normal. Urinalysis revealed a specific gravity of 1.029, and a 3 plus reaction for sugar. The blood nonprotein nitrogen was 19 and the sugar (fasting) 205 mg. per cent. The serum albumin and globulin were 3.8 and 1.6 Gm. per cent respectively. The serum cholesterol was 385, phosphorus 3.8, and calcium 11.0 mg. per cent. A phenolsulfonphthalein renal test revealed 35 per cent excretion of the dye in 15 minutes. On February 17, 1947, the glomerular filtration rate, corrected to a surface area of 1.73 square meters, was 108 cc./min. and the renal plasma flow 313 cc./min.; the filtration fraction was 0.35. The basal metabolic rate was minus 2 per cent. Lumbar puncture revealed an initial pressure of 330 mm. of water with a final pressure of 260 mm., after removal of 10 cc. of fluid. The serum electrolyte pattern is presented at the top of the next page.

X-ray examination revealed demineralization of the clinoid process of the sella turcica, arteriosclerosis of the pelvic blood vessels, moderate cardiac enlargement, and several callus-like areas on the left tenth and right fifth, eighth, and eleventh ribs in the axillary region. Intravenous pyelograms revealed no abnormalities. The diabetes mellitus was moderately severe; 50 units of insulin daily was necessary to control glycosuria when the intake of carbohydrate was 180 Gm. A Hickey-Hare test for diabetes insipidus (14) performed by Dr. Alfred Kahn on February 21, 1947, was negative. On February 19,

Date	CO ₂ combining power	Cl	Na	K ³	Comment
	mEq./l.	mEq./l.	mEq./l.	mEq./l.	
2/ 8/47	38.2	95			
2/15/47	34.8	97	143.7		
2/21/47	25.8	100	143.5	4.96	
2/25/47					Exploratory laparotomy
3/ 2/47	35.7	84			
3/ 4/47	38.4	90			
3/ 5/47					KCl, 4-8 Gm. per day, initiated
3/ 6/47	35.0	95			
3/10/47	28.4	95			
3/15/47	26.6	102			KCl therapy stopped.
3/24/47					"Salt clearance" test.
3/25/47	31.7	98	145.1		

1947, the urinary excretion of 17-ketosteroids was 4 mg. per 24 hours and that of sodium pregnanediol glucuronidate was 11 mg. per 24 hours.⁴

Exploratory laparotomy was performed on February 25, 1947, by Dr. Peter Heinbecker. The subcutaneous tissues were thin, and the muscles were thin and friable. The ovaries were atrophic. Nothing abnormal was found in the pancreas, kidneys, or adrenals. The patient manifested an extremely stormy postoperative course complicated by atelectasis, laryngitis, and partial wound disruption. She gradually improved and regained her preoperative state. The abdominal wound remained defective, in that tissue layers beneath the skin failed to unite.

F. B., Cushing's syndrome (Barnes Hosp. 141970): This 20-year-old white housewife had been under observation since December, 1946, when she complained of gradual weight gain, decrease in the amount of hair on her scalp and increase in the amount of hair on her face, purple striae, headaches, and amenorrhea; the duration of these symptoms was one year. A physician had noted hypertension. Examination revealed an obese girl, with a rounded, florid face. The skin showed many purple striae over the buttocks, thighs, breasts, and axillae. There were fat-pads over the neck, shoulders and hips. A moderate increase in hair was noticeable over the face, the shoulders, arms and legs. Acne was present on the face and upper arms. Male distribution of hair was evident over the pubic region. There were no abnormalities of the external genitalia. The visual fields were normal. The blood pressure averaged 150/105. Blood counts were normal.

³ Measurement of potassium was performed in the laboratory of Dr. Alexis F. Hartmann.

⁴ These measurements of steroidal excretion were made by Dr. Willard M. Allen. Ketosteroids were determined on the neutral steroid fraction obtained by hydrolysis of the butyl alcohol extract of urine, employing the metadinitrobenzene reagent. Glucuronidate was measured by the Allen-Viergiver method (15), which does not specifically identify the steroid as pregnanediol.

Urinalysis showed a few red cells and white cells per high power field in the sediment. Blood nonprotein nitrogen was normal as were the serum protein, calcium, phosphorus, cholesterol and phosphatase. A glucose tolerance test employing 1 Gm. of glucose per kilo. revealed the blood sugar (fasting) to be 80 mg. per cent; half-hour, 165 mg.; 1-hour, 215 mg.; 2-hour, 209 mg.; 3-hour, 105 mg.; and 4-hour, 39 mg. per cent. Additional chemical data follow:

Date	Serum CO ₂ combining power	Serum chloride	Serum sodium	17-ketos- teroid excretion in urine ¹	Sodium pregnanediol glucuronidate excretion ² in urine
	mEq./l.	mEq./l.	mEq./l.	mg./24 hrs.	mg./24 hrs.
1/17/47	28.1	96	139.8	24	33
4/12/48	30.6	106	145.2	27	45

A phenolsulfonphthalein renal test revealed 40 per cent excretion of the dye in 15 minutes. The urine was concentrated to a specific gravity of 1.032. On January 15, 1947, the glomerular filtration rate was 102 cc./min., renal blood flow 452 cc./min., filtration fraction 0.23 and Tm_{PAH} 66.0 mg./min., corrected to a surface area of 1.73 square meters. Lumbar puncture revealed an initial spinal fluid pressure of 260 mm. of water and a final pressure of 240 mm. The basal metabolic rate averaged minus 20 per cent. Intravenous pyelograms were normal. On January 21, 1947, an exploratory laparotomy was performed by Dr. Peter Heinbecker. The ovaries were found to be atrophic, but no other abnormalities were found in the pelvis, adrenals, kidneys, or pancreas. The post-operative course was uneventful. On April 8, 1947, a Hickey-Hare test for diabetes insipidus (14) was negative. Six days later the first "salt clearance" test here reported on this patient was performed.

In April, 1948, aside from a slight weight gain, her condition was essentially unchanged. The basal metabolic rate was minus 27 per cent. A second "salt clearance" test was performed on April 7, 1948. A Soffer test (4) on April 12, 1948, showed that the patient excreted 37.2 per cent of injected salt before administration of desoxycorticosterone acetate (DCA) and 17.5 per cent after administration of DCA; these findings were interpreted as a normal response.

L. P., Cushing's syndrome: Psychoneurosis (Barnes Hosp. 159194): This 16-year-old unmarried white girl, admitted on May 19, 1948, stated that one year before she had noted the onset of amenorrhea, increased appetite with a rapid weight gain of 60 pounds, and an increase in hair over the face and body; subsequently she noted pains in her extremities, occasional frontal headache and the appearance of lineae striae. She had been treated with desiccated thyroid and with injections, probably containing estrogenic material, until six weeks preceding admission; concomitantly with this therapy she began to bleed irregularly from the vagina. A right mastoidectomy had been performed at age of 10 months. Two sisters were said to have considerable facial hair; there was no family history of marked obesity. Examination revealed an obese girl weighing 190 pounds. The wrists and fingers were delicate. There was moderate increase in the hair of the torso and the sideburn area of the face. There was acne of the face. There were

TABLE 1. EFFECT OF INTRAVENOUS INFUSION OF 5 PER CENT SODIUM CHLORIDE SOLUTION UPON RENAL FUNCTION OF CONTROL SUBJECTS AND OF PATIENTS WITH CUSHING'S SYNDROME

Subject	Period	Renal excretory rates*				Plasma		Mannitol† U/P	Mannitol U/P†		Comment
		Water	Sodium	Chloride	Chloride	Clearance*			Chloride U/P		
						Mannitol	PAH				
F.M. ♀ 15 yrs. Convalescent from meningitis. Ht. 63 in.; wt. 105 lbs. S.A. 1.47 sq. m. Date 1/29/47	1	cc. per min. 3.28	mEq. per min. 0.461	mEq. per min. 0.269	mEq. per liter 102.1	cc. per min. 88.9	cc. per min. 534	26.9	33.5	5% NaCl 5% NaCl	
	2	3.57	0.400	0.320	104.7	104	680	28.5	34.5		
	3	3.59	0.514	0.363	100.8	103	656	28.7	31.1		
	4	4.27	0.691	0.526	112.6	110	796	25.7	23.5		
	5	4.98	1.04	0.786	116.6	118	821	23.7	17.6		
	6	6.35	1.34	1.10	116.4	120	850	19.0	12.8		
R.G. ♂ 50 yrs. Undiagnosed back pain Ht. 66.5 in.; wt. 125 lbs. S.A. 1.65 sq. m. Date 2/3/47	1	2.77	0.271	0.189	98.0	115	695	41.8	60.4	5% NaCl 5% NaCl	
	2	4.05	0.313	0.245	98.6	119	789	29.2	47.6		
	3	2.92	0.314	0.239	102.5	108	675	37.2	46.6		
	4	3.24	0.401	0.370	107.7	125	823	44.9	43.5		
	5	3.21	0.491	0.409	109.2	113	770	36.5	31.3		
	6	2.75	0.445	0.398	110.0	107	632	39.1	29.8		
	7	4.08	0.606	0.616	108.1	132	821	32.6	23.4		
	8	3.31	0.524	0.530	105.5	113	619	31.1	22.7		
J.K. ♂ 28 yrs. Normal. Ht. 70.5 in.; wt. 178 lbs. S.A. 2.00 sq. m. Date 2/10/47	1	6.26	0.472	0.337	97.1		648	29.2	28.1	5% NaCl	
	2	7.36	0.474	0.420	100.4	119	787	19.0	19.1		
	3	5.44	0.605	0.605	101.4	105	611	20.9	14.8		
	4	6.80	0.995	0.995	103.8	142	631	19.5	14.1		
	5	6.81	1.02	1.01	107.2	132	631				
	6	6.90	1.03	1.07	106.0		691				
G.S. ♀ 35 yrs. Cushing's syndrome. Ht. 59 in.; wt. 125 lbs. S.A. 1.52 sq. m. Date 3/24/47	1	4.32	0.365	0.281	98.6	114		26.3	40.0	5% NaCl 5% NaCl	
	2	4.40	0.331	0.327	99.3	118		26.6	35.9		
	3	8.10	1.17	0.908	106.6			15.44	14.73		
	4	13.9	2.45	2.18	117.0			10.14	7.604		
	5	13.0	2.45	2.18	117.6			10.34	7.154		
	6	8.91	1.60	1.47	111.0			13.34	9.194		
F.B. (1) ♀ 20 yrs. Cushing's syndrome. Ht. 64 in.; wt. 167 lbs. S.A. 1.82 sq. m. Date 4/14/47	1	3.24	0.319	0.319	102.2	127	553	39.6	41.8	5% NaCl 5% NaCl	
	2	3.15	0.287	0.287	102.4	131	600	42.6	47.5		
	3	5.45	0.748	0.748	106.4	147	610	27.1	21.0		
	4	11.0	2.01	2.01	112.3		895	17.84	11.04		
	5	10.1	2.00	2.00	112.5		790	20.84	11.84		
	6	7.55	1.57	1.57	111.9		761	21.14	13.14		
F.B. (2) ♀ 21 yrs. Cushing's syndrome. Ht. 64 in.; wt. 179 lbs. S.A. 1.88 sq. m. Date 4/7/48	1	2.39	0.568	0.160	102.2	81.5	416	31.1	51.8	5% NaCl 5% NaCl	
	2	2.74	0.318	0.190	102.1	91.0	416	31.3	49.0		
	3	3.49	0.500	0.362	105.1	89.6	495	25.5	25.8		
	4	7.30	1.41	1.25	111.2	104	675	14.3	9.24		
	5	7.81	1.62	1.47	112.1	105	535	13.1	7.94		
	6	6.32	1.28	1.20	111.9	115	535	18.2	10.7		
L.P. ♀ 16 yrs. Cushing's syndrome. Ht. 69 in.; wt. 188 lbs. S.A. 2.02 sq. m. Date 5/28/48	1	5.07	0.680	0.628	106.4	92.8	443	18.3	15.8	5% NaCl 5% NaCl	
	2	4.34	0.586	0.528	105.3	81.9	397	19.5	16.8		
	3	5.32	0.798	0.707	108.7	81.5	397	15.9	13.0		
	4	6.42	1.13	1.03	113.9	86.2	429	13.1	9.14		
	5	6.45	1.19	1.10	114.6	83.8	399	13.0	8.78		
	6	6.97	1.26	1.18	114.7	97.1	426	13.9	9.40		

* Corrected to a standard body surface area of 1.73 square meters.

† For explanation of these ratios, see text.

‡ S.A. signifies body surface area.

§ Maximum values only, due to analytical difficulties; true figure is lower.

pinkish striae on breasts, arms and legs. The breasts were flat but about 10 cm. in diameter. The highest recorded blood pressure was 158/96 on admission; subsequent daily readings averaged 120/70. Dr. Willard M. Allen noted no abnormalities of the pelvic organs other than slight enlargement of the clitoris and a small erosion of the cervix which bled after manipulation; later, during her stay in the hospital, the patient bled slightly from the vagina. Blood counts were normal. The urine contained a trace of reducing substance in two of four random specimens. Plasma protein and serum calcium, phosphorus, and alkaline phosphatase concentrations were at normal levels. The plasma carbon dioxide combining power was 26.9 mEq./l.; the plasma chloride level was 103 mEq./l., and that of cholesterol 338 mg. per cent. Oral administration of 1 Gm. of glucose per kilo produced the following blood sugar curve: fasting, 105 mg. per cent; half-hour, 129 mg.; 1-hour, 208 mg.; 2-hour, 222 mg.; and 3-hour, 167 mg. per cent. Reducing substance appeared in the urine during the test. A glucose-insulin tolerance test conducted by Dr. W. Perry, involving the simultaneous oral administration of 1 Gm. of glucose per kilo and 0.1 unit of regular insulin per kilo intravenously, affected the blood sugar as follows: fasting, 77 mg. per cent; 20 minutes, 80 mg.; 30 minutes, 84 mg.; 60 minutes, 133 mg.; 90 minutes, 159 mg.; and 120 minutes, 167 mg. per cent. The basal metabolic rate averaged minus 3 per cent. Renal phenolsulfonphthalein excretion was 20 per cent in 15 minutes. After lumbar puncture, the initial pressure of the spinal fluid was 190 mm. of water. The 24-hour urinary excretion of 17-ketosteroids was 8.0 mg. and that of sodium pregnanediol glucuronide 31.0 mg.⁴ X-ray examination of the skull revealed hyperostosis frontalis interna; the sella was normal. Intravenous pyelograms revealed no indication of abnormalities of kidneys or adrenals. A psychiatric consultant found evidence of a mixed type of psychoneurosis.

As suggested by Dr. Willard M. Allen, this patient might well be classified not as an example of full-blown Cushing's syndrome, but as an example of "pseudo-Cushing's syndrome"—a term applied by Dr. Allen to patients manifesting obesity, amenorrhea, and hirsutism as the essential clinical abnormalities. However, this patient exhibited, in addition, the diminished tolerance to glucose administration characteristic of Cushing's syndrome and appeared resistant to the hypoglycemic action of insulin.

RESULTS

Chloride excretion—Chloride excretion rates in 3 experiments on 3 control subjects and in 4 experiments on 3 patients with Cushing's syndrome are presented in Table 1 and Figure 1. Prior to the injection of 5 per cent sodium chloride, chloride excretion rates were approximately the same for all subjects save L. P.; the latter patient manifested a higher rate of excretion. Following the start of the injection, 2 patients with Cushing's syndrome (G. S. and F. B.) exhibited a much more pronounced and prompt chloride diuresis than did the control subjects. Excretion of sodium paralleled that of chloride (Table 1).

Water excretion—Concomitant with the marked chloride excretion observed in 2 of the patients with Cushing's syndrome, there was observed increased urine flow (Table 1 and Figure 2). The urine flows in the control subjects and in 1 patient with Cushing's syndrome (L. P.) did not change markedly during the injection of hypertonic salt solution.

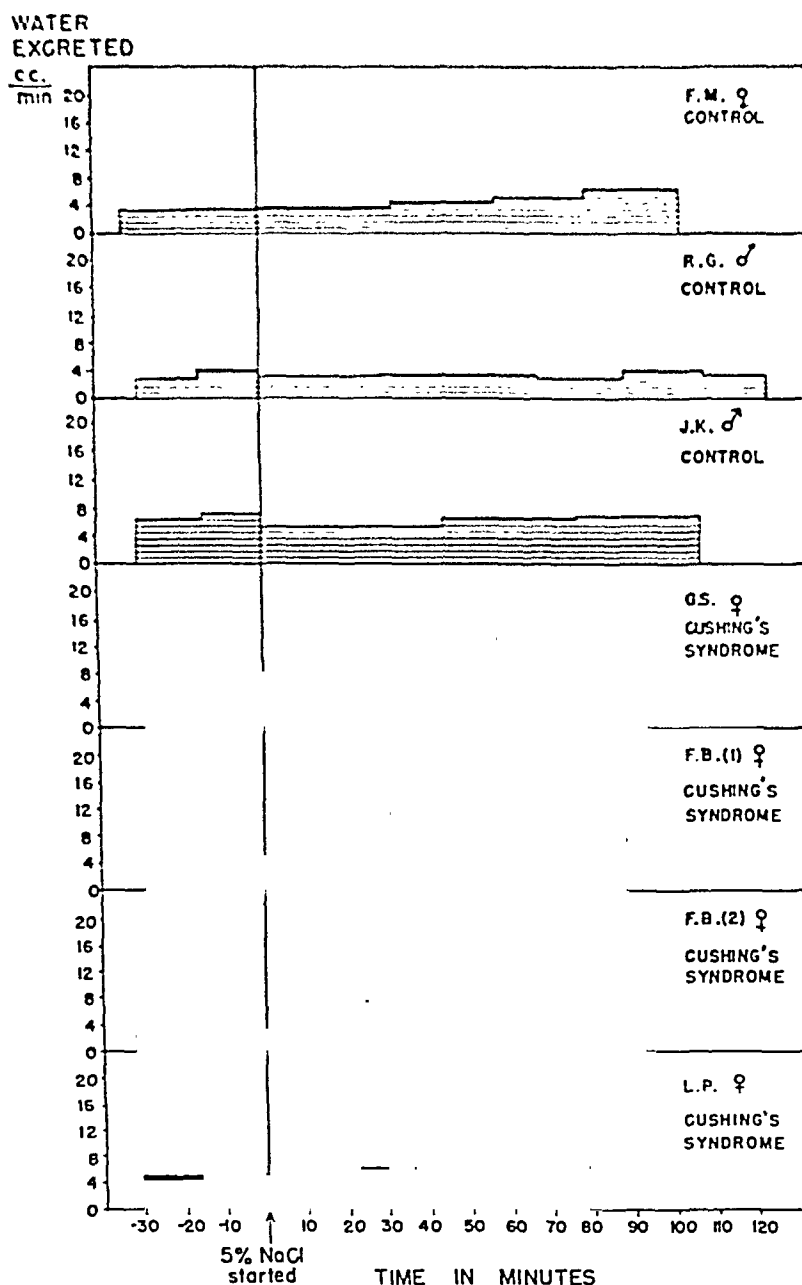


FIG. 2. Rate of renal excretion of water before and after the administration of hypertonic sodium chloride, corrected to a standard body surface area of 1.73 square meters.

normally low in patient G. S. (see case report) and probably also in L. P.; both of these patients manifested Cushing's syndrome. There was a tendency for both the mannitol and PAH clearances to rise following the injec-

tion of salt, but these changes were inconsistent and of about the same magnitude in the controls as in experimental subjects. For example, while the glomerular filtration rate in the second experiment on F. B. rose after the injection of salt, a rise of equal magnitude was observed in control subject F. M. It seemed unlikely, therefore, that increases in glomerular filtration rate in the patients with Cushing's syndrome could have been the only factor causing the excessive excretion of salt.

Tubular reabsorption of water and chloride An attempt is made here to express chloride excretion rates at comparable rates of glomerular filtration and urine flow in order to ascertain if the observed differences in excretion may be ascribed to differences in degrees of tubular reabsorption. On the assumption that mannitol is not reabsorbed from the tubules, we have

employed the ratio $\frac{\text{urine mannitol concentration}}{\text{plasma mannitol concentration}}$, designated as "man-

nitol U/P," to represent the degree to which the glomerular filtrate is concentrated by the reabsorption of water (Table 1). The higher the ratio, the greater the degree of water reabsorption. As a corollary, any substance which is partially reabsorbed by the tubules (such as chloride) is characterized by a U/P ratio less than the simultaneous mannitol U/P ratio, the discrepancy being dependent on the amount reabsorbed. Hence, the ratio

$\frac{\text{mannitol U/P}}{\text{chloride U/P}}$ is always greater than 1.0; the higher the ratio, the greater

the degree of reabsorption of chloride. This method of expressing degree of chloride reabsorption has the advantage that correction is made for differences in urine flow and glomerular filtration.

$\frac{\text{Mannitol U/P}}{\text{Chloride U/P}}$ ratios throughout all clearance periods for 5 subjects are

presented in Table 1 and Figure 3. Analytical difficulties with the mannitol determination prevent inclusion of all values for control J. K. As would be expected, this ratio declined in all subjects after the injection of salt. It will be noted, however, that the values for the ratio in the patients with Cushing's syndrome (G. S. and F. B) dropped precipitously after the start of the salt injection and soon reached low levels never attained by the control subjects. We interpret these findings as indicating an abnormally pronounced diminution of chloride reabsorption in the renal tubules of these 2 patients with Cushing's syndrome when a large "salt load" was imposed.

In the third patient, L. P., the ratio $\frac{\text{mannitol U/P}}{\text{chloride U/P}}$ was initially relatively

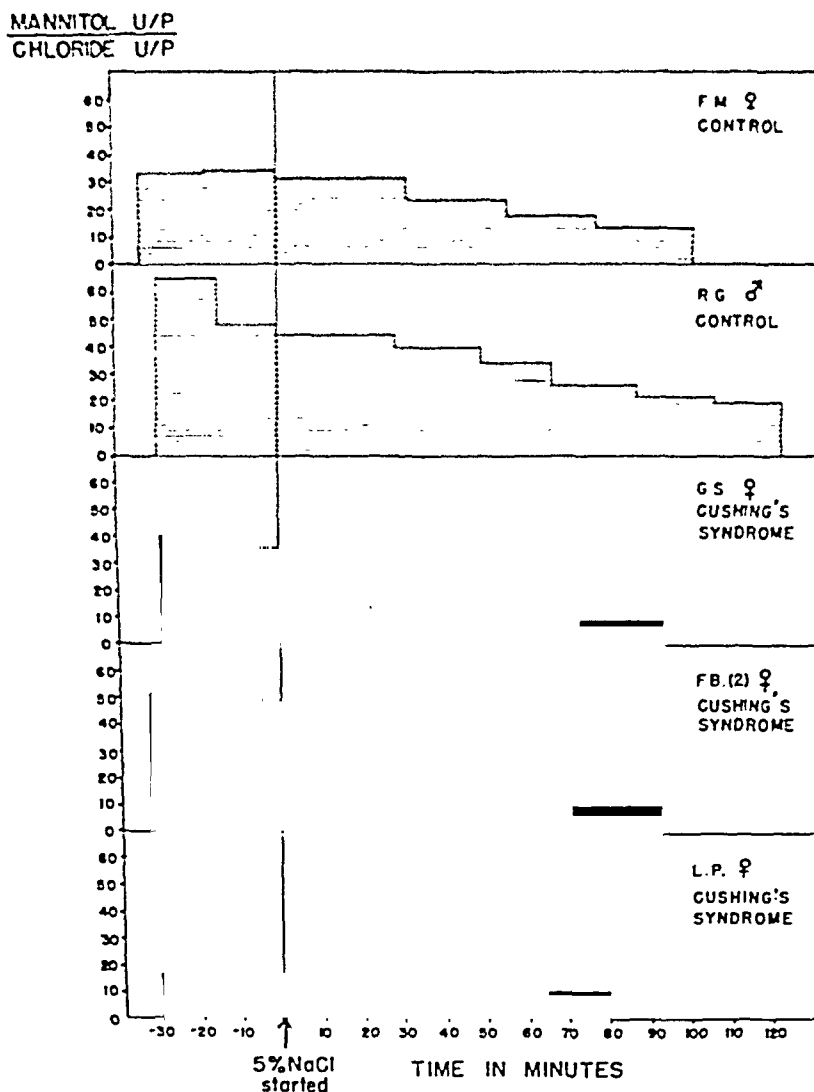


FIG. 3. Chloride reabsorption ratios before and after the administration of hypertonic sodium chloride. The ratios in periods 4, 5, and 6 in the experiment on G. S. and in the first experiment on F. B. are to be regarded as maximum values and higher than the true figures.

low and consequently its subsequent fall to very low levels appeared less striking.

It will be noted (Table 1) that the mannitol U/P ratio observed in the patients with Cushing's syndrome became considerably lower after the salt was administered as compared to the pre-injection levels. In contrast, this ratio in the control subjects showed only slight decreases or no change as compared to the pre-injection levels. We interpret these findings as indi-

cating that the injection of hypertonic salt resulted in an abnormally pronounced diminution of water reabsorption in the renal tubules of the patients with Cushing's syndrome.

DISCUSSION

Our findings resemble those of Soffer *et al.* (4) in suggesting that under certain circumstances some patients with Cushing's syndrome excrete sodium chloride (salt) in larger amounts than do control subjects. Soffer has suggested that one possible explanation for the increased excretion of salt by his patients with Cushing's syndrome given DCA, may be conversion of the injected DCA into a non-salt-retaining, or "salt-excreting" hormone. Since at least 2 of our 3 patients with Cushing's syndrome exhibited a relatively increased diuresis of salt under conditions *not* involving administration of exogenous DCA, such an explanation for our findings is impossible. We favor the possible explanation, for our findings, that in Cushing's syndrome there is increased production of hormones of the 17-hydroxycorticosterone type, which, though predominantly "carbohydrate-active," also have "salt-excreting" effects (17), presumably through an inhibition of renal tubular reabsorption of salt. Evidence supporting such increased production is the report that in Cushing's syndrome there occurs an abnormally high urinary excretion of substances thought to be 21-carbon adrenocortical steroids having a ketone or hydroxyl group on carbon atom 11 and an hydroxyl group on the 17th carbon atom (18). The influence of naturally produced salt-retaining hormones may, therefore, be overbalanced in these patients.

In patient F. B., with Cushing's syndrome, administration of 10 mg. of DCA according to Soffer's technique (4) resulted in a "normal" salt-retaining response despite the fact that she exhibited salt diuresis during our clearance study performed five days previously. The patient had been fed a measured diet supplying 8 Gm. of sodium chloride daily for two days before the test was initiated. In this instance, it is possible that the salt-retaining effect of 10 mg. of DCA was sufficient to overcome any salt-excreting effect of the patient's own adrenal hormones. Soffer (19) has also found that some patients with Cushing's syndrome give a normal response to the test. A similar variability of response of patients with Cushing's syndrome to salt-excretion tests is exemplified by the results recorded for our patient L. P. This patient differed from our other 2 abnormal subjects in that the output of chloride in the control period was greater; and probably, in part as a result of this, the responses to the injection of hypertonic sodium chloride solution were less striking. As an explanation for such variations in response, the possibly significant effect of variations in salt intake preceding the experiments cannot be excluded. Additional study of this factor in both normal subjects and in patients is desirable. Further-

more, it is not to be expected that all patients classified as manifesting "Cushing's syndrome" are identical in their physiologic status. Thus, no two of the three patients referred to herein as manifesting the syndrome appeared either on gross inspection or after laboratory investigation to manifest exactly the same disease-complex.

We have further observed in 2 obese females with arterial hypertension that the Soffer tests was positive for Cushing's syndrome at the time of menstruation and negative in the same patients in the intermenstrual period.⁵ The 2 patients had subsisted on a measured diet supplying 8 Gm. of salt daily for at least six days before each test. During the intermenstrual and premenstrual periods, the urinary excretion and presumably the blood concentration of estrogenic substances is relatively great, and at these times in the sexual cycle there occurs a retention of salt and water (20). Salt retention has also been demonstrated to occur following the administration of estrone, α -estradiol, and progesterone (21). At the onset of the menses, excretion of substances of ovarian and luteal origin decreases precipitously and there occurs a concomitant increase in excretion of sodium and chloride (20). In contrast to the marked cyclical fluctuations in the urinary excretion of estrogenic substances, the excretion of "carbohydrate-active" corticosteroids is relatively constant throughout all phases of the sexual cycle (22). Hence, during the catamenia, the ratio

$$\frac{\text{blood corticosteroid content}}{\text{blood estrogen content}}$$

is probably greater than at any other time during the sexual cycle. Such a temporary preponderance of adrenocortical influence may possibly account for the observed positive response to a test for hypercorticoadrenalism such as the Soffer test. With the rise in estrogen production which occurs after menstruation, the hormonal interrelationships may be so adjusted that the salt-retaining effects of the estrogenic and luteal hormones predominate over the salt-excreting effects of the corticosteroids; at this time the Soffer test might be expected to be negative. It is impossible for us to explain why DCA produced the observed *increased* excretion of salt in Cushing's syndrome (4) and during menstruation in our 2 obese subjects with hypertension.

One of our patients with Cushing's syndrome (G. S.) who excreted injected salt unusually rapidly, exhibited marked abnormalities in the serum electrolyte pattern resembling those previously described by Willson, Power and Kepler (23). Another patient with Cushing's syndrome (F. B.)

⁵ These 2 patients were hospitalized during the course of an investigation of hypertension supported by the U. S. Public Health Service. We are indebted to Dr. Henry A. Schroeder for the privilege of studying them.

excreted injected salt in a similar manner, although she exhibited a normal serum electrolyte distribution.

Two of our patients with Cushing's syndrome (G. S. and F. B.) underwent a test for diabetes insipidus, performed according to the method of Hickey and Hare (14). Both manifested a normal antidiuretic response to 2.5 per cent sodium chloride solution. Chronically diminished output of the antidiuretic factor of the posterior lobe of the pituitary, therefore, probably does not account for the diuresis of water and chloride which we observed. In this connection, it is of interest that the test of Hickey and Hare resembles in many aspects the procedure which we have described under "Methods," save that we did not establish the marked diuresis in the control periods which their test (14) entails.

As we have already indicated, the diminished degree of chloride and water reabsorption observed in at least 2 of our patients with Cushing's syndrome is very possibly due to an imbalance of adrenal hormones or hormonal byproducts influencing the renal tubular epithelium. Although we regard this as the most likely explanation, we have no incontrovertible evidence that this mechanism actually occurs. Another explanation, which we regard as less likely, is that the co-existing hypertension and slight renal ischemia in the patients with Cushing's syndrome so altered tubular function as to prevent a normal degree of reabsorption of chloride. We also cannot completely exclude the possibility that increases in glomerular filtration rate and consequent increased rate of flow of the glomerular filtrate down the tubules may have been partially responsible for the observed diminution in the degree of tubular reabsorption of chloride.

SUMMARY AND CONCLUSIONS

1. The renal excretion of chloride, water, mannitol and para-aminohippurate has been measured before, during, and after the rapid intravenous injection of a "salt load" of 20 grams of sodium chloride in 3 controls and in 3 patients with Cushing's syndrome.

2. Following the injection of sodium chloride, the rate of excretion of chloride, sodium, and water by 2 of the 3 patients with Cushing's syndrome exceeded that observed in the control subjects.

3. The increased excretion of chloride and water by 2 of the 3 patients with Cushing's syndrome was accompanied by a diminished degree of renal tubular reabsorption of these substances as compared to the controls.

Acknowledgments

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GRAVES' DISEASE: TREATMENT WITH RADIOIODINE (I^{131})* †

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CARRIER-FREE I^{131} has been administered by us to patients with Graves' disease since August 1944 in order to study primarily: a) the efficiency of I^{131} as an agent employed to destroy hyperfunctioning thyroids subtotally; and b) the uptake of iodine by the thyroid before and after those symptoms of hyperfunction of the thyroid associated with Graves' disease have been relieved (1).

Up to the time of writing this summary, 68 patients with Graves' disease have been studied and sufficient data are available to permit reporting results in 46 patients. All these patients had undoubted Graves' disease varying in degree from mild to severe; the majority had moderately enlarged thyroids, moderate hyperthyroidism and (with one exception) no nodules in the thyroid. In the initial phases of this study, small (250 microcuries) doses of radioactive iodine were given at weekly intervals; later more adequate single doses of 1000 to 4000 microcuries were given and repeated as necessary up to a total dose as high as 10,411 microcuries.

Prior to the studies on humans, the effects of I^{131} were studied in animals. Three hundred microcuries per kilogram were injected subcutaneously into rabbits and dogs. At the end of ten days, the rabbit thyroids showed extensive necrosis, hemorrhage, polymorphonuclear infiltration and arterial changes. By the twentieth day other rabbit thyroids still had polymorphonuclear plus eosinophilic and lymphocytic infiltration, healing vascular changes and fibrosis. At the end of thirty and forty days, further fibrosis

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had occurred; the thyroid had decreased approximately 50 per cent in size; and arteries had intimal and medial fibrosis, thickening and perivascular fibrosis. Only a few questionably normal acini remained at the poles. Dog thyroids removed forty days after 300 microcuries per kilogram of I^{131} showed similar changes and after one hundred days the thyroid of one dog was practically completely destroyed. One rabbit showed damage in the renal tubules but the other glands of internal secretion, the trachea, bone marrow, liver, spleen, lymph nodes, kidneys and peripheral blood counts were normal (2). Barker (3) in recent but unpublished work has not found this degree of change in the thyroids of rats sacrificed three to six days after being given 350 microcuries per kilogram of I^{131} ; the explanation though not yet clear probably is related to the amount of iodine in the diet of his animals and a lower uptake of iodine by their thyroids. More recently (4) rabbits were given 500 microcuries of I^{131} per pound orally and five days later up to 4000 microcuries per pound orally and were sacrificed at the end of four weeks. The rabbits' thyroids were partially or completely destroyed and in addition, tracheal and renal tubular injury were apparent; no other damage was demonstrable from gross or microscopic examination of tissues. Gorbman (5) has used doses of 3-4 and up to 50-55 millicuries of I^{131} in mice and found 90 per cent destruction of the thyroid and 100 per cent destruction of the parathyroids at the end of one hundred and twenty days even with the lower dosage. Bengt Skanse (6) has shown that doses of I^{131} (50 microcuries or less) definitely decrease the ability of the chick thyroid to take up iodine. It should be pointed out that the largest single dose of I^{131} (measured as microcuries per Kg. of body weight) administered by us to a patient with Graves' disease is one-fourth as large as that required to destroy subtotally the thyroids of normal rabbits (without injuring other tissues) and one-fortieth the dose that destroys the parathyroids of normal mice. As regards total dosage to patients, one-seventeenth of the dose that destroys the parathyroids of mice is the largest amount given to thyrotoxic patients at the University of California.

UPTAKE STUDIES

The maximum amounts of I^{131} taken up by the thyroids of 48 patients with Graves' disease varied from 12.5 to 92 per cent of an orally administered dose before any treatment, with an average of 61 per cent. The lowest figure was found in a patient in partial remission. In patients treated with Lugol's solution or with one of the thiouracils or with both, the uptake varied from 22 per cent to 70 per cent and averaged 48 per cent.

In order to determine the uptake of radioiodine per gram of thyroid tissue, the size of the thyroid was estimated by palpation. In the past it has been the custom in the Thyroid Clinic of the University of California to

palpate the thyroid carefully preoperatively, record the weight as estimated by the clinician and compare this weight with that of the thyroid tissue removed at operation. The same individual (M. H. S.) who has estimated the weights in patients treated with radioiodine has also examined enough thyroids in surgically treated patients to obtain some appraisal of his accuracy. This was done as follows: surgeons doing subtotal thyroidectomies measured the amount of thyroid tissue left *in situ* after a Halsted type of operation by cutting a piece of the removed thyroid tissue equal in size to the tissue left in the patient. The total removed tissue was weighed during the gross examination of the specimen by the pathologist and the weight of the small amount of tissue corresponding to that left in the patient was added to give the total weight of the patient's thyroid. This actual weight has been compared with the clinically estimated weight in 74 patients with toxic goiters. The results of this study indicate that this particular observer (M. H. S.) has in two-thirds of these 74 patients ranged in his estimates from 24 per cent below the actual weight to 13 per cent above; 95 per cent of his estimates ranged from 42 per cent below to 31 per cent above the actual weights. In terms of weight of thyroid tissue, the error in 66 per cent of patients varied from 13 grams below, to 7 grams above the measured weight; while in 95 per cent of the patients the range of error was between 23 grams below and 17 grams above the confirmed weight. While this degree of accuracy was disappointing, it nevertheless probably represents the accuracy to be expected from a conscientious observer. In only 25 per cent of patients were the estimates within 10 per cent of the actual weight.

The weight of the thyroid and the amount of radioiodine in the thyroid must be known in order to determine the radiation to the thyroid. Also obvious is the necessity of recognizing errors in: a) the measurement of the millicurie, b) the measurement of the uptake of radioiodine by the thyroid *in situ*, and c) the estimation of thyroid size. The latter errors have received too little attention. The methods used in measuring the millicurie and the uptake of radioiodine *in situ* have been described previously (1).

The concentration of I^{131} per gram of thyroid tissue was calculated in 44 patients, and ranged from 21.3 to 247.8 microcuries per gram with an average of 77.7 microcuries per gram. Twenty-seven of these patients were in the group classified as having had a satisfactory response, yet the variability of uptake was tremendous since the lowest uptake measured 21.3 and the highest 143.5 microcuries per gram of thyroid with the average at 95.2 microcuries. One patient who was not normal at the end of a year after a total dose of 9750 microcuries had an uptake of 247.8 microcuries per gram. At the other extreme, two patients who developed frank myxedema had an uptake of 43.5 and 47.5 microcuries per gram respectively.

Thus the evidence presented indicates that many factors other than the concentration of I^{131} are active in determining the amount of I^{131} needed to produce a satisfactory remission in a patient with Graves' disease.

RESULTS

A patient is considered to have responded satisfactorily to treatment by I^{131} if within approximately four months the signs and symptoms of thyrotoxicosis have disappeared, the thyroid has returned to normal size and the basal metabolic rate, level of serum protein-bound iodine and other laboratory findings are within normal limits. Forty-two patients fall into this category. The data are summarized in Table 1. Complete data on the first 25 of these patients have been published (1); the added 17 patients differ in no material way from the original 25.

TABLE 1. SATISFACTORY RESULTS OF I^{131} THERAPY IN 42 PATIENTS WITH GRAVES' DISEASE (4/16/48).

	Before treatment	After treatment
Basal metabolic rate	plus 27.7%*	minus 6.7%
Protein-bound iodine (serum)	9.7 micrograms %	6.2 micrograms %
Estimated weight of thyroid	29 grams	12 grams
Total dose I^{131} : 3017 microcuries		
Time to return to normal: 3.35 months		

* Mean values.

Four patients fail to satisfy the criteria for a satisfactory response in the sense that the interval between the beginning of therapy and the return to normality was prolonged, as shown in Table 2. These four patients had larger thyroids, more severe hyperthyroidism (as measured by their clinical

TABLE 2. SLOW RESULTS OF I^{131} THERAPY IN 4 PATIENTS WITH GRAVES' DISEASE (4/19/48).

	Before treatment	After treatment
Basal metabolic rate	plus 47%*	minus 4%
Protein-bound iodine (serum)	12.9 micrograms %	5.8 micrograms %
Estimated weight of thyroid	38 grams	15 grams
Total dose I^{131} : 4838 microcuries		
Time to return to normal: 14.5 months		

* Mean values.

status, basal metabolic rates and serum protein-bound iodine) and required larger doses of radioiodine as well as a longer time to return to normal.

In addition to the 46 patients described in Tables 1 and 2, two more were subjected to subtotal thyroidectomy. One was done because of poor co-operation and the other because of a poor response to radioiodine therapy and subsequent evidence that she had a nodular goiter. Three more patients have not returned to normal and eventually if and when they do return to normal, will be classified as "slow responses"; five have not returned to the clinic sufficiently often nor recently enough to allow adequate appraisal. One more has become pregnant so that determination of her status in any precise way is difficult. Finally, 11 of our 68 patients have been followed for too short a time after therapy to allow proper evaluation at this date. The slow results and failures can be accounted for in the main by our too conservative dose schedules in the early days and inability to obtain enough radioiodine to give as large doses as were thought necessary.

Some of the clinical and laboratory findings in these patients were of interest. Not infrequently tenderness in the thyroid region was noted within twenty-four to seventy-two hours after I^{131} had been given. It was sometimes noted spontaneously by the patient and was nearly always apparent on palpation, especially when 2000 or more microcuries were given in a single dose. During this period of tenderness in the thyroid, the blood sedimentation rate increased to as high as 35 mm. (Wintrobe); also the protein-bound iodine rose temporarily and was sometimes associated with an increase in the signs and symptoms of hyperthyroidism between the fourth and tenth days.

The eyes of 47 patients were measured with a Hertel (Zeiss) ophthalmometer in order to record changes in prominence during and after radioiodine therapy. There was no significant change in 31 patients. In 12 patients an increase of 1.5 to 2.0 millimeters was noted. In 4 individuals the increase in exophthalmos was greater than 2 millimeters and actually was 5 millimeters in one; three of the four were classified as having severe progressive exophthalmos. The 3 patients with severe exophthalmos were treated with thyroid to tolerance, and in one there was a regression of 1 millimeter in the right eye and 2 millimeters in the left eye after a period of nine months. Thus 66 per cent of patients showed no change in the prominence of their eyes, 25.5 per cent showed a definite but minimal increase and 8.5 per cent had a marked increase. In our experience an increase in exophthalmos occurs less frequently than in surgically treated patients and more frequently than in patients treated with x-ray. The significance of these findings must await further experience.

Fifteen patients who had had Graves' disease had uptake studies repeated after therapy with I^{131} . The results in eleven of these patients were

within the normal range, with an average uptake of 16.6 per cent of a test dose of I^{131} (range: 2.5 to 25 per cent). Four patients who had been treated and who were in the lower normal range as regards their thyroid status, yet who could not be defined as having frank hypothyroidism, showed an interesting phenomenon in that their thyroids took up 3, 46, 49, and 75 per cent respectively of a test dose of I^{131} . A possible explanation of the high uptake in 3 of these patients is that so much of the thyroid had been destroyed that the small remnant was overfunctioning in the sense of utilizing more iodine in an attempt to produce enough thyroid hormone to maintain the patient in a normal thyroid state.

COMPLICATIONS

Two patients became myxedematous after therapy. The first was a woman with only moderate hyperthyroidism whose basal metabolic rate was plus 26 per cent and serum protein-bound iodine 9.6 micrograms per cent, whose estimated 25 gram thyroid took up 49 per cent of her therapeutic dose of 2250 microcuries of I^{131} . The second patient was a woman with somewhat more marked hyperthyroidism with a basal metabolic rate of plus 38 per cent and serum protein-bound iodine of 9.9 micrograms per cent, whose 35 gram thyroid took up 75 per cent of her therapeutic dose of 4000 microcuries of I^{131} . Both patients developed myxedema a few weeks after therapy, in the same manner as does the patient with treated myxedema who exhibits symptoms and signs of recurring myxedema when thyroid therapy is discontinued. The doses of I^{131} of only 43.5 and 47.5 microcuries of I^{131} per gram of thyroid respectively were apparently large enough to inhibit completely the production of thyroid hormone. Not enough time has elapsed to permit us to be sure that the destruction of the thyroids in these two patients is complete and permanent or whether some function will be regained in the future.

COMMENT

Disagreement still exists among groups of workers using I^{131} regarding selection of patients for therapy, dose to be employed and the advisability of a single large dose rather than several smaller doses (7, 8). Differences of 30 to 50 per cent or more in the measurement of aliquot fractions of I^{131} persist from one laboratory to another. Only time will permit resolution of some of these problems and even then there will remain different but adequate doses and dose schedules.

SUMMARY

The results of treatment of 46 thyrotoxic patients with radioiodine over the period from 1944 to 1948 are presented, in which there were 42 satis-

factory responses and 4 unsatisfactory responses. The latter are felt to be due to a conservative approach to therapy in the early phases of the study.

Animal experimentation has been carried on to determine the effects of massive doses of I^{131} upon tissues surrounding the thyroid, and the pathologic changes have been described. It has been found that upwards of forty times the maximum therapeutic dose (in microcuries per kilogram) used in humans is required to produce serious damage in contiguous structures in mice and rabbits.

The uptake of radioiodine by the thyroid in untreated Graves' disease was found to average 61 per cent, whereas in the treated patient it averaged 16.6 per cent. There appears to be a paradoxical lack of correlation between clinical response to treatment and the number of microcuries per gram delivered to the thyroid.

I^{131} in doses of 2 millicuries or more causes clinical and laboratory changes that point to tissue destruction (thyroid). After therapy with I^{131} exophthalmos progresses less than in patients treated by subtotal thyroidectomy and more than in patients treated with x-ray. Two patients in this series developed myxedema.

CONCLUSIONS

Radioactive iodine (I^{131}) in adequate dosage will destroy subtotally the hyperfunctioning thyroids in patients with Graves' disease and thereby produce usually a satisfactory remission of the symptoms and signs of hyperthyroidism. Further studies ultimately will disclose its place in the therapy of Graves' disease.

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THE DEVELOPMENT OF DIABETES MELLITUS IN ADDISON'S DISEASE

CASE REPORT WITH AUTOPSY

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THE occurrence of both adrenal cortical insufficiency and diabetes mellitus in the same individual is rare; reports of only twenty-two such instances are available. Each of these diseases is associated with abnormalities in carbohydrate metabolism in some respects antagonistic to the other and consequently the simultaneous occurrence of the two is of considerable interest.

In the reports reviewed, diabetes was established in the majority of the patients before the development of Addison's disease. (1-12). In four, however, both diseases were recognized simultaneously (13-16), while in three others, (17-19) the adrenal insufficiency antedated the appearance of diabetes.

The presence of adrenal cortical hypofunction frequently alters the co-existing diabetic state. A number of these patients are reported to exhibit an unusual sensitivity to insulin (1, 3, 5, 8, 13, 14, 15, 18, 19) such as is characteristically found in Addison's disease alone. Severe hypoglycemic reactions are commonly mentioned and, in those patients with pre-existing diabetes, a fall in insulin requirement frequently follows the development of hypoadrenalism (4, 6, 8, 9, 10). However, in one instance, the daily dosage of insulin is reported unchanged (7), following the appearance of adrenal insufficiency and in ten others, either insufficient evidence is presented to establish this point or no reference is made to this problem.

The effect of adrenal replacement therapy on the coexistent diabetic state varies with the substances employed. When desoxycorticosterone acetate (DCA) has been used, no change has been reported. This is consistent with experimental evidence indicating that this steroid plays a negligible role in carbohydrate metabolism. On the other hand, the available evidence indicates that extracts of the whole gland and more strikingly the C-11 oxy-steroids may lead to an increased glycosuria and possibly greater insulin requirement in the diabetic Addisonian patient. The first report suggesting this action of the whole gland extract is Levy-Simpson's in 1932 (14). His patient entered the hospital in a state of adrenal insufficiency with hypoglycemia and the underlying diabetic state became ap-

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parent only after nineteen days of adrenal cortical extract therapy. However, the increased food intake due to control of the adrenal insufficiency may also have been a factor here. Later, Bloomfield (4), Bickel (8) and Bowen, Koepf, Bissell and Hall (6) noted higher blood sugars during fasting when their patients were receiving extracts of whole adrenal gland than when DCA was employed, and Soffer (12) thought that the insulin requirement of his patient was greater under similar circumstances. Thorn and Clinton (18) and Sprague and co-workers (11) observed a marked increase in glycosuria and nitrogen excretion following the administration of 11-dehydro-17-hydroxycorticosterone (compound E), and in addition the latter authors reported similar though less striking responses to hog adrenal extract (lipo-adrenal cortex) and in one instance to 11-dehydrocorticosterone (compound A).

The present report concerns a third patient in whom diabetes mellitus with striking insulin sensitivity appeared two and one-half years *after* the diagnosis of Addison's disease was established.

CASE HISTORY

E.G. was a 22-year-old single woman who was first admitted to the Presbyterian Hospital in July 1942 for evaluation of suspected Addison's disease. Her initial symptoms appeared six years previously, at which time she began to have bouts of nausea and vomiting occasionally accompanied by fainting during her menstrual periods. It was not until three years later that increasing pigmentation of her skin became evident, and in the ensuing year she became aware of increasing fatigability and a decrease in appetite which led to a 7-pound weight loss. One year before admission to this hospital she went to the Mayo Clinic, where a Cutler-Power-Wilder test, though somewhat equivocal, was on the whole in keeping with the diagnosis of Addison's disease. Subsequently the addition of 10 grams of sodium chloride a day to her diet led to a definite improvement in her symptoms.

There was no history of known exposure to tuberculosis. It is of interest that a paternal aunt died of diabetes.

Physical Examination: On admission in July of 1942, she was a thin, intelligent young woman who looked chronically ill. There was a diffuse muddy pigmentation of the skin, a few inky black moles, and there were bluish spots on the gums. Her blood pressure was 95/65, temperature 99.8° and pulse 84. The heart was not enlarged; the regular rhythm was interspersed with runs of extrasystoles. The remainder of the physical examination revealed nothing abnormal.

Initial laboratory findings: Hemoglobin 14.5 Gm. per 100 cc.; red blood cells 4,360,000; white blood cells 5,850; polymorphonuclears 52 per cent, lymphocytes 38 per cent, monocytes 4 per cent, eosinophiles 5 per cent, basophiles 1 per cent; erythrocyte sedimentation rate 17 millimeters in one hour.

The urine specific gravity was 1.017; there was no albumin, glucose, acetone or diacetic acid and no formed elements microscopically.

X-ray examination showed a normal chest and skull and no evidence of calcification in the adrenal area.

A tuberculin test was negative in a 1:100,000 dilution; the basal metabolism was

minus 14 per cent; 24-hour excretion of 17-ketosteroids in the urine was 1.5 mg.; and an electrocardiogram showed no evidence of heart muscle damage.

The serum nonprotein nitrogen was 37 mg. per cent and total protein, 6.2 Gm. per cent; blood sugar (fasting), 86, 80 and 74 mg. per cent on three separate occasions; serum carbon dioxide content, 27.4 mEq. per liter; serum chlorides, 100.6 mEq. per liter. The calculated concentration of serum sodium was 138 mEq. per liter, and the direct gravimetric serum sodium, 137.7 mEq. per liter.

Course: When placed on a regimen of salt restriction, she lost 2 kilograms in five days and became anorexic, weak and dizzy. The blood pressure fell to 75/55 and the serum sodium (gravimetric) fell to 132.5 mEq. per liter. With this evidence for adrenal cortical insufficiency she was then given desoxycorticosterone acetate (DCA), by subcutaneous

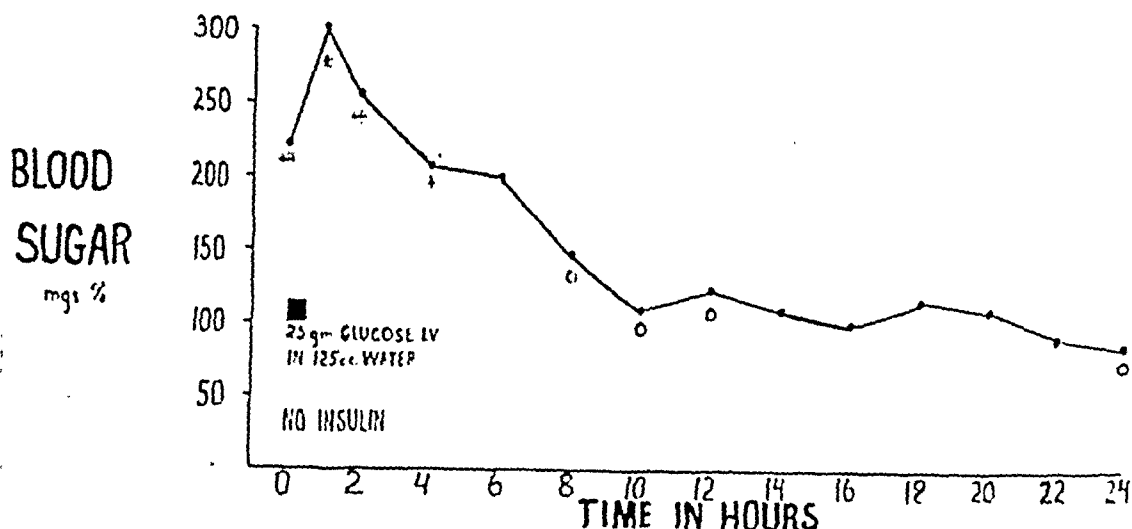


FIG. 1. Blood sugar curve following an intravenous glucose-tolerance test with subsequent 24-hour fast. Urine glucose (O to +++) at 0, 1, 2, 4, 8, 10, 12 and 24 hours is shown directly beneath corresponding blood sugar values.

injection. In a week and half she gained 3 kilograms and was discharged taking 4 mg. a day without added sodium chloride.

Over the next fifteen months her general condition was satisfactory and serum electrolyte values were maintained within normal limits on the above dosage with an unrestricted diet to which 4 grams of sodium chloride was added daily in the form of enteric-coated tablets. A postprandial blood sugar, three months after discharge, was 67 mg. per cent. Twelve months later, she reported she had had a weak spell associated with mental confusion, which responded promptly to administration of orange juice. Because of this her blood sugar was again checked two months after this episode and was, surprisingly, found to be 308 mg. per cent. With this she had a severe glycosuria (4 plus) though no acetonuria.

In the ensuing three months she was admitted to the hospital twice for regulation of the diabetes and during this time the following studies were done: Initially she was given a diet containing 140 Gm. carbohydrate, 80 Gm. protein and 65 Gm. fat. She received no insulin and the daily amount of glycosuria was determined. On day one, 71 Gm. of glucose was excreted; on days three and four, 94 and 91 Gm. respectively. A

blood sugar, taken during fasting, on the sixth morning of this regimen was 174 mg. per cent. In spite of the severity of the glycosuria, a 24-hour fast without insulin resulted in a disappearance of both glycosuria and hyperglycemia (Fig. 1).

In attempting regulation of the diabetes it was found that extraordinarily small amounts of insulin were sufficient to control the heavy glycosuria and that an additional 1 or 2 units resulted in severe hypoglycemic episodes. Thus, 4 units of standard insulin given before breakfast and before supper was sufficient to maintain adequate control of the diabetes on a diet of 190 Gm. carbohydrate, 100 Gm. protein and 80 Gm. fat.

During this 3-month period, her clinical condition remained good and, with the exception of a drop in sodium to 131.9 mEq. per liter during the period of severe glycosuria, her serum electrolytes remained normal, and the maintenance dose of 3 to 5 mg. of DCA plus 2 to 4 Gm. of sodium chloride was continued.

After this, her third admission, she remained in fairly good health for the next two years, though never strong enough to do more than help out with the lighter chores at home. She maintained the same diet and her insulin requirements varied from 2 to 6 units twice daily. In order to reduce the number of daily injections, her adrenal disease was controlled during this period with subcutaneous implantation of pellets of DCA, inserted at approximately yearly intervals. In the course of these two years she was hospitalized five times for intercurrent infections or for DCA pellet implantations.

Her final, and ninth, admission was in January 1946 for an acute illness of twelve hours' duration beginning with sore throat, weakness, chills and fever. Her temperature on arrival was 103.2°, pulse 100, respirations 18 and blood pressure 96/55. She was acutely ill, lethargic and dehydrated with evidences of a mild upper respiratory infection. The pharynx was not acutely inflamed. Aside from the previously observed pigmentary changes, the rest of the physical examination was entirely normal.

Subsequent laboratory findings: Hemoglobin 12 Gm. per 100 cc.; red count 3,390,000; white count 17,000; polymorphonuclears 75 per cent, lymphocytes 21 per cent, monocytes 3 per cent, basophiles 1 per cent; erythrocyte sedimentation rate 60 mm. in one hour.

Urinalysis showed 4 plus glucose, acetone and diacetic acid, but was otherwise normal.

The blood sugar level (fasting) was 444 mg. per cent. The serum carbon dioxide content was 23.1 mEq. per liter; serum chlorides 95.4 mEq. per liter; serum urea nitrogen 11 mg. per cent; the calculated serum sodium 128.5 mEq. per liter. A throat culture showed hemolytic streptococci but the blood culture showed no growth.

The course of her disease during this admission, with essential laboratory data and therapy, is outlined in Figure 2. When seen initially she was in a state of acute adrenal insufficiency and from this aspect her condition continued to be precarious for the first seventy-two hours, with profound shock, hyperpyrexia and accompanying decreased concentration of serum sodium. During this period she developed generalized muscular rigidity, sufficiently marked in the nuchal region to indicate a lumbar puncture. The results, however, showed a normal spinal fluid. To combat the adrenal insufficiency, she was given both DCA and aqueous whole adrenal extract as well as supportive measures in the form of whole blood, plasma and saline infusions. After the first twenty-four hours the use of the intravenous route was curtailed because she developed signs of fluid retention, namely, facial edema, enlargement of the liver, gallop rhythm, an increase in pulse rate to 120, a rise in venous pressure, rapid respirations (rate 40) and x-ray evidence of pulmonary congestion. Because of these findings she was digitalized on the second day and most of the fluids were subsequently administered by hypodermoclysis.

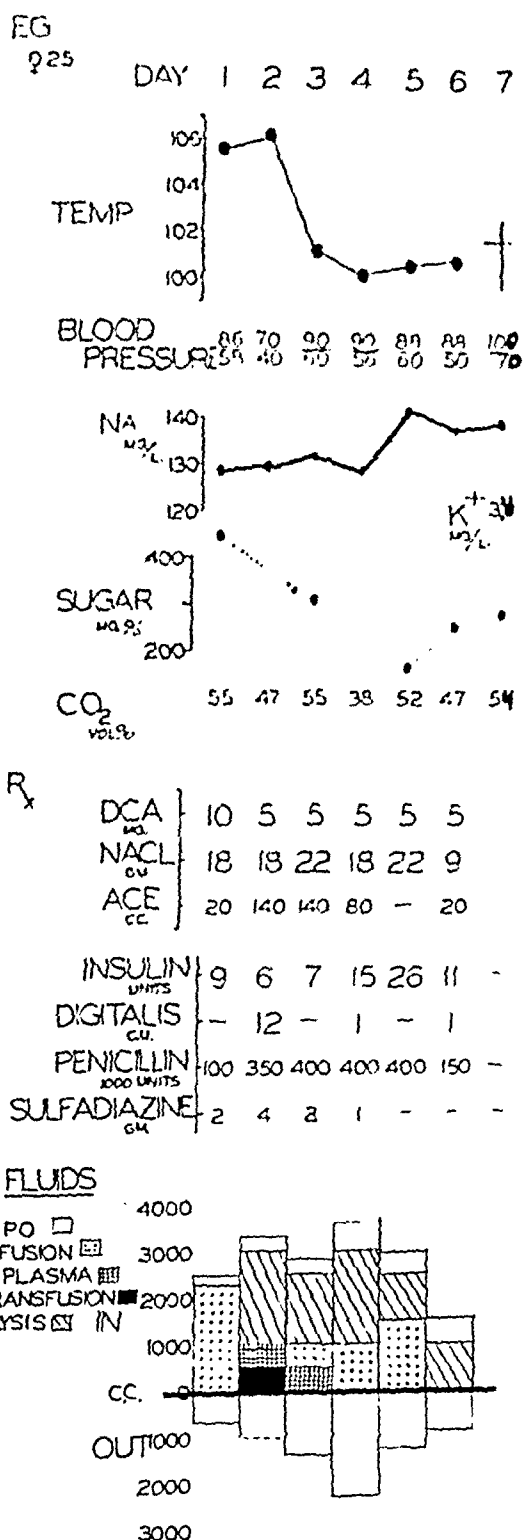


FIG. 2. Chart of clinical and laboratory findings and of therapy employed during final admission of patient E. G.

DCA = desoxycorticosterone acetate

ACE = 10% alcoholic extract of whole adrenal gland.

Her streptococcus infection was treated with both penicillin and sufficient sulfadiazine to maintain an adequate level of this substance in the blood.

On the third day she improved strikingly, but within the following twenty-four hours the diabetes, which on admission had not presented difficulties, became a major problem and she developed moderately severe acidosis. In the first three days no attempt had been made to control the diabetes rigidly, since hypoglycemia seemed the major danger in view of the acute adrenal insufficiency and negligible food intake. Extreme insulin sensitivity persisted; on the first day a total of 9 units effected complete clearing of both acetoneuria and glycosuria. Subsequently insulin was given cautiously, in doses of 3 or 4 units, according to the amount of sugar in the urine. On the fourth day, in spite of this, heavy glycosuria persisted and acetoneuria appeared with a sharp drop in carbon dioxide content of the serum to 16.2 mEq. per liter. This acidosis cleared with the administration of only 26 units of insulin. At no time during this admission were any hypoglycemic episodes observed.

After the fourth day, the primary problems were pulmonary congestion and the appearance of cardiac arrhythmia. By the fifth day the venous pressure had risen to 195 mm. and x-ray examination of the chest on the following day showed further increase in the pulmonary vascular markings with either bilateral hydrothorax or widespread edema in both lower lobes. A part of the picture was attributed to the fall in serum total protein from the initial level of 6 Gm. to a level of 4.5 Gm. per cent on the fifth day. As to the cardiac arrhythmia, extrasystoles were heard at intervals on the third day. During the fifth night she had a short episode of syncope. The next morning an electrocardiogram revealed varying degrees of A-V block, with low voltage, premature ventricular beats and a marked prolongation of the QT interval. Some of these abnormalities suggested hypocalcemia or hyperkalemia or both. The following night there was a second episode of syncope, with convulsive movements accompanied by asystole. In spite of the repeated administration of adrenalin, four more similar attacks occurred over the next twelve hours; in the last of these, she died.

Final laboratory findings: On the morning of death the blood sugar (fasting) was 278 mg. per cent and the following values were found in serum: carbon dioxide, 22.7 mEq. per liter; chlorides, 106.2 mEq. per liter; urea nitrogen, 6 mg. per cent; calculated sodium, 138.9 mEq. per liter; and potassium, 3.4 mEq. per liter. The urine showed glucose 4 plus and acetone 1 plus, but no diacetic acid.

AUTOPSY

(four hours post mortem)

Gross

The pleural and peritoneal cavities each contained 300-400 cc. of clear fluid. The mesenteric and systemic abdominal veins were distended. The mesenteric and retroperitoneal tissues were more moist than normal. The heart was not enlarged or dilated. It weighed 270 Gm. The myocardium was normal. A small amount of clear fluid was expressed from the cut surfaces of the lungs which weighed 470 and 360 Gm. respectively. No gross abnormalities could be seen in the spleen, liver, gall bladder or kidneys. The pancreas was small, but its normal lobular structure was preserved.

The right adrenal was a gray, wafer-like structure measuring $3 \times 18 \times 0.3$ mm. The left consisted of two 1-2 mm. nodules situated in a thin sheet of fibrous tissue.

A small subserous cyst of the right ovary was filled with clear, yellow fluid. The thyroid was small and its cut surface was gray. Colloid appeared to be reduced. Four

parathyroid glands weighed 143 mg. The bone marrow was slightly pale. The thymus measured $2.5 \times 0.8 \times 0.5$ cm.

Microscopic

Heart: In a few small areas of the interventricular septum, the interstitial spaces were widened and the muscle fibers narrowed. There was no necrosis, inflammation or scarring of the myocardium or of the conduction bundle.

Lungs: The interlobular septa, the peribronchial tissue, and the alveoli were mildly edematous.

Liver: The sinusoids in the central zones were mildly congested. Many portal areas were infiltrated by lymphocytes, wandering cells, and polymorphonuclear leukocytes. In sections stained with Best carmine for glycogen, the cytoplasm of liver cells contained an abundance of carminophilic granules, especially in the periportal zone.

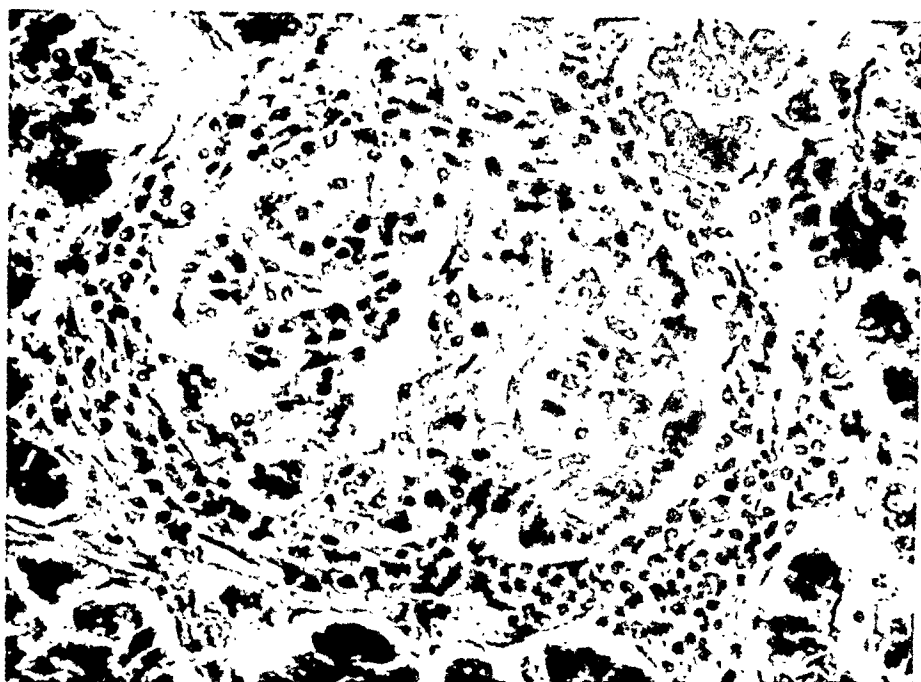


FIG. 3. Pancreas. Tissue around an islet of Langerhans is infiltrated by inflammatory cells. $\times 360$.

Pancreas: Tissue blocks were taken from ten areas. There were small focal areas of infiltration by lymphocytes, and occasional polymorphonuclear leukocytes and fat-laden phagocytes in five. In one section the inflammatory cells infiltrated an islet, (Fig.3). The islets were rather sparse and shrunk. Their cells were very small. The cytoplasm consisted of a very thin rim of dense acidophilic material around small, occasionally pyknotic nuclei. There was no hydropic degeneration, fibrosis, or hyalinization of the islet tissue. Sections from seven blocks fixed in Bouin's solution were stained with chrome hematoxylin phloxin by Dr. George Gomori, of the University of Chicago. No granules were found in any of these (Fig. 4A). Alpha, beta, and delta granules were seen in abundance in islets in sections of pancreas from another case used as a control for the staining technique (Fig. 4B).

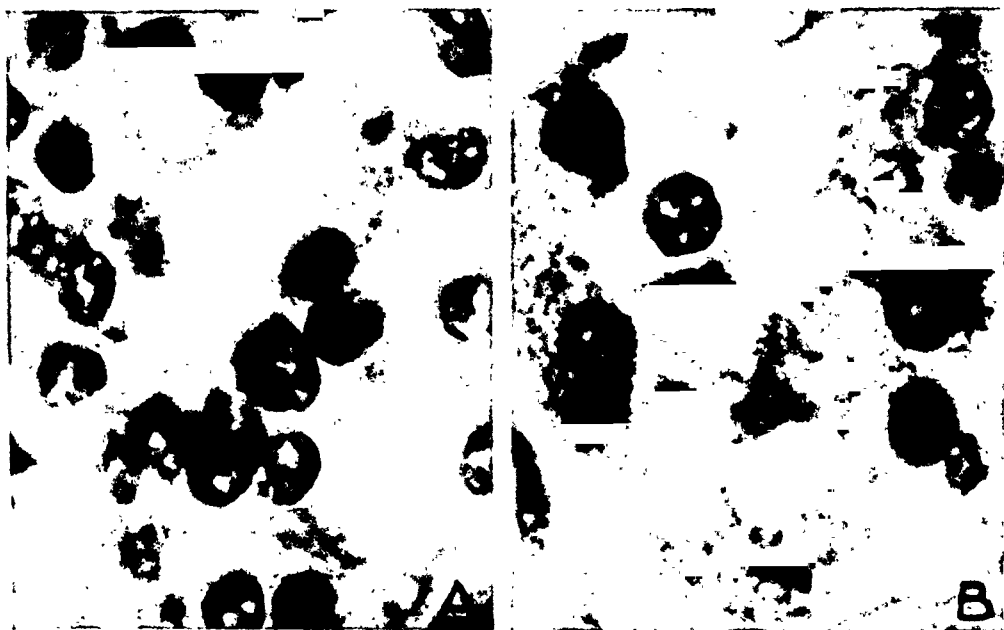


FIG. 4. Cells of the islets of Langerhans. Chrome-hematoxylin-phloxin stain. $\times 980$. A). From the pancreas of patient E. G. No alpha, beta or delta granules are seen in the cytoplasm.

B) From a normal pancreas serving as a control. Granules are abundant.

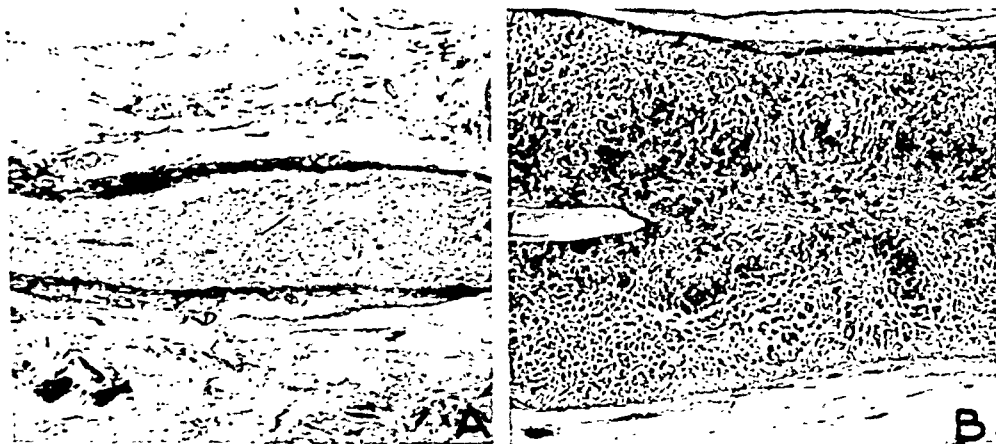


FIG. 5. A) Right adrenal from the patient of this report. Atrophy is advanced. Medullary tissue is absent. B) A normal adrenal for comparison, at the same magnification. $\times 16$.

Adrenals: The right adrenal was about two-thirds of a high power field in thickness (Fig. 5). Within a collapsed, thickened capsule, the tissue consisted of fibrous tissue, collapsed stroma, and a few small groups of atrophied cortical cells, focally infiltrated by lymphocytes. Residual tissue of the left adrenal was even less conspicuous. No medullary tissue was seen in either gland.

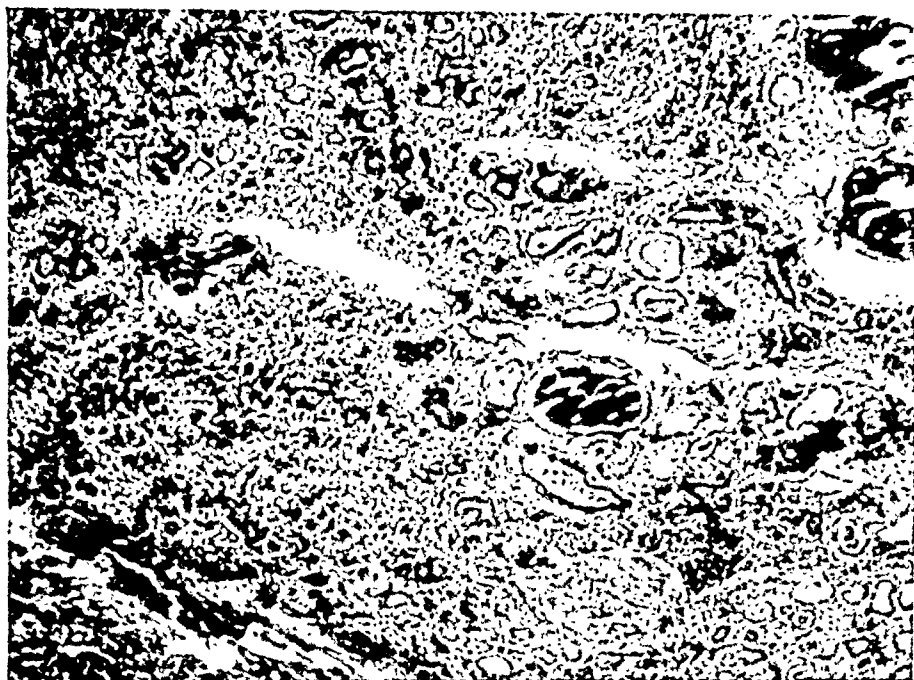


FIG. 6. Thyroid. Atrophy of the follicles in areas infiltrated by lymphocytes. $\times 80$.

Kidneys: There were no abnormal findings. In sections stained with Best carmine, a small amount of carminophylic granules was present in the straight segments of the proximal convoluted tubules and in Henle's loops.

Ovary: This contained a mature Graafian follicle and a corpus luteum cyst.

Uterus: There was cystic glandular hyperplasia of the endometrium.

Thyroid: The major portion of the gland was normal. However, the follicles were atrophied in scattered areas where the tissue was infiltrated by lymphocytes which were occasionally oriented in small follicles (Fig. 6).

Parathyroid glands: These were normal.

Thymus: The thymus was essentially normal. The corticomedullary structure could be distinguished.

Breasts: The glandular tissue was normal. There was marked periacinar fibrosis.

Hypophysis: There was an unusually small number of chromophobe cells, with an apparently normal proportion of acidophiles and basophiles, although the latter were sparsely granulated. There was no frank necrosis, but the presence of pyknosis of nuclei, confluence of cells, breakdown of cell walls, widespread vacuolization of cytoplasm and clumping of cytoplasmic granules in certain areas, adjacent to normal and active cells, was evidence of some degeneration. The presence of such cytologic details as the Golgi

apparatus in the normal cells indicated that the degenerative changes in adjacent cells were probably actual rather than artifacts.

Skin: Melanin was increased in the basal and germinal cell layers.

Brain: (Dr. Abner Wolf) There were no changes of note in the frontal gyrus, the hypothalamus, or in the medulla.

DISCUSSION

The development of diabetes mellitus following Addison's disease is a physiologic paradox of which only three instances other than this patient have been reported. It may be termed a paradox because the expected changes resulting from adrenal cortical insufficiency are in the direction of increased carbohydrate utilization due to the removal of the inhibiting effects of the cortical hormones upon this phase of metabolism. Ingle (20) has established that injections of cortical substances result in a decreased ability to utilize fed glucose. The more recent work of Cori (21) has related this finding to one specific step in carbohydrate breakdown--the conversion of glucose to glucose-6-phosphate--and has conclusively shown that adrenal extracts, in conjunction with anterior pituitary extracts, exert an inhibitory effect on this reaction. This inhibition is in turn blocked by insulin. Furthermore, since the classical studies of Long and Lukens (22), it has been known that the removal of the inhibitory effect of the adrenals on glucose utilization or on gluconeogenesis by adrenalectomy greatly ameliorates the diabetic state in a previously pancreatectomized animal. It is therefore, somewhat of a paradox that this patient, two and one-half years after known adrenal insufficiency existed, should have developed severe diabetes to the extent of a glycosuria of nearly 100 grams daily.

An unusual sensitivity to exogenous insulin, such as this patient exhibited, has been remarked upon in a number of the previous reports of this dual disease picture and is, of course, known in uncomplicated Addison's disease. It has, too, its experimental counterpart in the adrenalectomized, pancreatectomized animal and is also observed after hypophysectomy. Explanations of this sensitivity, taking into account what is known of insulin action and of "anti-insulins," remain unsatisfactory. The action of insulin described by Cori does not, as he himself has pointed out, explain this observed sensitivity. Recently, in addition to its role in glucose utilization in the liver, a peripheral site of insulin action has been suggested by Perlmutter and Greep (23). These authors reported that the addition of insulin led to an increased glucose uptake in the striated muscle of hypophysectomized as well as of normal rats. Whether the insulin sensitivity in this patient can be related to such a peripheral action of insulin can not be said at present, since the effect of adrenalectomy on this action has not yet been examined.

In all, the explanation of insulin sensitivity such as this patient, in common with other similar cases, exhibited remains unsatisfactory. Recent observations (24, 25) suggest that in addition to the pituitary and the adrenal so-called "anti-insulins," an anti-insulin may be elaborated by the pancreas. It is conceivable that the total absence of granules in the pancreatic islets in the patient here described might indicate the lack of the normal anti-insulin of this region, and in part account for her sensitivity to exogenous insulin. Against this argument are the histologic findings on one other patient with diabetes mellitus and Addison's disease, recently reported by Bernstein (26). In Bloomfield's (4) original description of this patient a marked fall in insulin requirement was noted when Addison's disease supervened. At autopsy Bernstein observed, in Gomori-stain specimens of the pancreas, that "alpha cells were easily recognizable, but only a few unequivocal beta cells were present."

SUMMARY

1. The fourth case of Addison's disease with subsequent appearance of diabetes mellitus is reported.

2. The patient developed diabetes two and one-half years after the diagnosis of adrenal cortical insufficiency was established.

3. A severe degree of glycosuria (71 to 94 Gm. per day) existed in the absence of insulin therapy; however, this glycosuria was controlled with a total of only 6 to 8 units of insulin a day and extreme sensitivity to minute changes in dosage was observed. A 24-hour fast resulted in disappearance of the diabetic state.

4. Autopsy showed: a) complete absence of alpha, beta and delta granules in the islets of the pancreas and unusually small islet cells, and b) marked atrophy of both adrenals with associated lymphocytic infiltrations in the thyroid and liver.

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SURVEY OF A SCOTTISH DIABETIC CLINIC

A STUDY OF THE ETIOLOGY OF DIABETES MELLITUS

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DURING the present century statistical information has frequently been gathered from patients attending diabetic clinics, mostly in America. The value of these surveys depends chiefly on the extent to which they can be used in studying the etiology of diabetes mellitus. Greater knowledge of the circumstances under which diabetes occurs in man will permit the important advances which have recently been made in the experimental production of diabetes in animals to be placed more readily in their proper perspective. In carrying out a survey of diabetics seen at a Scottish hospital we have accordingly limited ourselves to a consideration of factors associated with the onset of the disease, *viz.* the incidence of diabetes according to age and sex, the frequency of diabetes in married and unmarried women, the body weight of diabetics prior to the onset of the disease, the blood pressure of diabetics, the hereditary factor in diabetes, and the association of diabetes with other endocrine and nonendocrine diseases. The data have been treated statistically. Means are given with their standard errors, and probabilities of less than 0.05 have been accepted as significant.

CLINICAL MATERIAL

The survey is based on the clinical records of 1309 cases of diabetes seen at the outpatient department and in the wards of the Victoria Infirmary, Glasgow during the 10-year period from the inception of a metabolic clinic early in 1932 until February 1942. The following data are relevant to this clinical material:

a) Situation of the hospital. The infirmary serves southern Glasgow and neighbourhood, an industrial area whose inhabitants have an age distribution slightly different from that of Scottish people as a whole: in 1931, 31.3 per cent of men and 31.8 per cent of women in Glasgow were 40 years of age or older, as compared with 32.3 per cent and 34.6 per cent respectively for Scotland. There are also minor racial differences between Glasgow and the rest of Scotland. In this connection only persons of either Jewish or Irish origin need be considered, since a high incidence of diabetes is known

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to occur among them (1, 2). The size of the Jewish community cannot seriously affect the issue, for only 4.8 per cent of our diabetic cases were Jews. The last census of Scotland (1931) revealed that 2.58 per cent of the Scottish population had been born in Ireland, whereas in Glasgow 4.81 per cent were Irish-born. Thus any differences that may exist between the inhabitants of Glasgow and Scottish people as a whole cannot be important, and we shall later present evidence which suggests that our cases are probably a representative sample of Scottish diabetics.

b) Diagnostic criteria. In most cases the diagnosis of diabetes has been based on a typical history supported by a single blood sugar determination made at random on the patient's first visit to the hospital. Doubtful cases were investigated by a glucose tolerance test (50 Gm. glucose orally): using Hagedorn and Jensen's technique on capillary blood, diabetes was considered to be present when the peak of the curve exceeded 190 mg. glucose per 100 ml. of blood and the return to the fasting level was delayed. Some clinicians have suggested higher blood-sugar values as critical levels for the diagnosis of diabetes: thus Joslin (1) considers 200 mg. per 100 ml. to be the significant level and Hale-White and Payne (3) suggest 220 mg. for elderly subjects. These differences of opinion are unlikely to vitiate our data, for few of our cases lie within the doubtful zone. In a random sample of 269 case records we found that only 5 per cent of diagnoses had been based on blood-sugar values of less than 200 mg. per 100 ml. of blood and 13 per cent on values of less than 220 mg. (these include single estimations taken at random as well as tolerance-curve peaks).

c) Representative nature of the clinical material. In an attempt to test whether our cases were a representative sample of Scottish diabetics, we compared the ratio of male to female patients at our clinic with the ratio of male to female deaths from diabetes recorded in the official returns of deaths for Scotland. In using mortality statistics for this purpose, it is necessary to show i) that the mortality statistics do not distort the proportion in which male and female diabetics die, and ii) that the ratio of male to female deaths from diabetes remains reasonably constant during the period studied. It can then be assumed that the sex ratio among new cases is the same as the sex ratio found in the mortality statistics, despite the long interval elapsing between onset of diabetes and death.

The first of these conditions was shown to be fulfilled when the death certificates of 128 of our diabetic cases were traced. Although only 67 per cent of these were finally classified as deaths due to diabetes in the Annual Returns of the Registrar-General for Scotland¹, the sex distribution was

¹ This reduction is due to losses of two kinds, a) failure by practitioners to enter diabetes on the death certificate, and b) other coincident diseases taking precedence over diabetes as the classifying cause of death.

very little altered (Table 1). In order to test the second point, the standardized death rates² from diabetes during the period 1901-1940 were calculated for men and women separately (Fig. 1). Although the ratio of the female to the male death rate was subject to considerable disturbances up

TABLE 1. AN INVESTIGATION INTO THE CERTIFICATION AND RECORDING OF DEATHS FROM DIABETES IN SCOTLAND
[The certificates and mortality data are classified according to the 1929 revision of the International List of Causes of Death.]

	Total number	Ratio of female to male diabetics in groups	χ^2 * test
Number of investigated deaths from diabetes	128	2.12:1	
Those classified as deaths from diabetes in the Annual Returns of the Registrar-General for Scotland	86 (67%)	2.19:1	
Annual number of deaths from diabetes recorded in the Returns of the Registrar-General for Scotland (mean for the years 1931-40)	781	2.06:1	
Therefore, estimated true number of deaths from diabetes occurring annually in Scotland	1163	2.00:1	} 3.67†
New cases seen in clinical practice (authors' clinic, 1932-42)	1309	2.26:1	

* Chi-square, an index of dispersion.
† Sex distribution at clinic not significantly different from sex distribution of deaths among Scottish diabetics during 1931-40.

to 1930, it attained a rather stable plateau after that year. It is therefore probable that the similarity between the sex distribution at our clinic and the sex distribution of deaths among Scottish diabetics during 1931-40

² The standardized rate is appropriate here because it allows for changes in the average age of the population during the period studied. On account of the long duration of diabetes in recent years, we have used broad age groups (0-14, 15-34, 35-54 and 55 years upwards) in calculating the standardized rate, which is based on a population of a million persons distributed as was the population of Scotland in 1901.

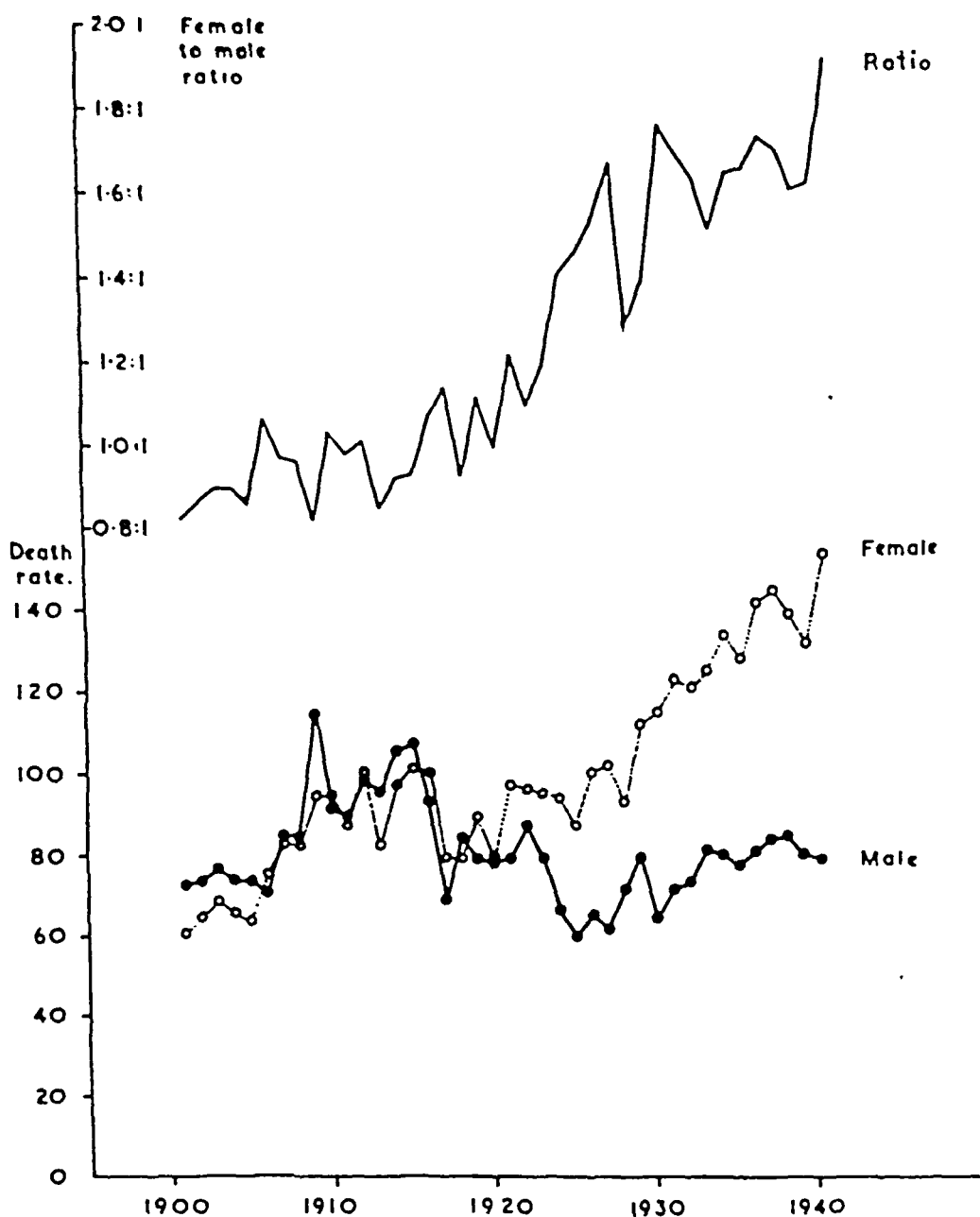


FIG. 1. Standardized death rate from diabetes in Scotland (1901-1940).

(Table 1) is an indication of the representative nature of our clinical material.

Comparisons have also been made (Table 2) between the sex incidence noted at some other clinics and the sex distribution of deaths from diabetes

in the local mortality statistics. The sex incidence noted at these clinics varies from an excess to a deficiency of females but the observations are sometimes quite at variance with the ratio of female to male deaths recorded in the local returns of deaths. Whilst complete reliance cannot be placed on this use of the crude mortality statistics (*cf.* Joslin and Lombard

TABLE 2. THE SEX INCIDENCE OF DIABETES AT CERTAIN DIABETIC CLINICS

Author	Locality	Cases seen at clinic		Local deaths from diabetes
		Total number	Female to male ratio	Female to male ratio*
<i>American:</i>				
Altschul and Nathan (5)	Harlem, N.Y.	639†	4.0 :1	3.04:1† (New York, 1937)
		106‡	1.8 :1	2.09:1‡ (New York, 1937)
John (6, 7)	Cleveland 1921-27	1986	1.14:1	1.42:1 (Ohio, 1921-27)
Joslin (1)	Boston			
(i)	1898-1933	9853	1.13:1	1.54:1 (Massachusetts, 1901-32)
(ii)	1936-38	2887	1.28:1	1.92:1 (Massachusetts, 1933-38)
Wendt and Peck (8)	Detroit 1919-29	1073	1.83:1	1.36:1 (Michigan, 1919-29)
Wilder and Browne (9)	Mayo Clinic			
(i)	1924-28	1889	0.87:1	1.31:1 (Minnesota, 1924-28)
(ii)	1935-37	1998	0.77:1	1.30:1 (Minnesota, 1935-37)
<i>British:</i>				
Cambridge (10)	London	1000	0.67:1	1.42:1 (London, 1925)
Murray Lyon (11)	Edinburgh 1924-32 (approx.)	1700	1.82:1	1.81:1 (Scotland, 1924-32)
Present authors	Glasgow 1932-42	1309	2.26:1	2.06:1 (Scotland, 1931-40)

* Not adjusted for distortion of ratio in the process of certifying and recording deaths.

† Negro cases.

‡ White cases.

(4)), these comparisons do suggest that some clinics are not representative of the local diabetic population.

d) *The frequency of undiagnosed diabetes in the population of Scotland.* It should be pointed out that we have shown our diabetics to be representative only of *diagnosed* diabetes in the population of Scotland. It is quite probable that the type of diabetic in whom the disease goes undiagnosed is different from the average case of diabetes. Undiagnosed cases will tend to be mild and without definite symptoms. Furthermore, there is no reason to believe that lack of symptoms will affect male and female diabetics to the same extent. For example, genital pruritus was the chief complaint in 26 per cent of our female cases but in only 0.8 per cent of our male cases; it is conceivable that a number of these females would not have come for

diagnosis, were it not for the pruritus. It would therefore seem important to estimate the extent to which diabetes goes undiagnosed at different ages in the Scottish population.

In the case of young adults, a direct approach to the problem of the true number of diabetics in the population is possible through the use of data accumulated at the examination of army recruits. We have therefore obtained an analysis of the medical records of 413,110 consecutive male and female Scottish recruits. The ages covered were 17-45 years for men and

TABLE 3. DIABETES IN SCOTTISH ARMY RECRUITS

	Males	Females
Number of recruits examined	342,091	71,019
Average age (years)	27.1	21.7
Range (years)	17- 45	17-35
Diabetics found	594	26
Diabetics per thousand examined	1.74	0.37
American National Health Survey rate, in population of similar age composition [†] (diabetics per thousand)	1.0	0.7
χ^2 test for diabetics found compared with number expected on the basis of the American National Health Survey	48.15*	7.58*

* The number found differs significantly from the number expected.

17-35 years for women and the only classes of the population exempted from military service were persons working in essential industries and women with young families. When glycosuria was found in the routine testing of urine, the test was repeated on the following day; if sugar were still present, the recruit was sent to a specialist for a glucose tolerance test and clinical assessment. The difficulties and decisions of the specialist are well illustrated by the paper of Peel and Peel (12) on diabetes among Scottish army recruits, but the important feature of the data which we have obtained is that it rests ultimately on clinical judgment of each individual case.

The incidence of diabetes in Scottish recruits is shown in Table 3. In order to compare this incidence with frequency of diabetes diagnosed in the ordinary course of events, we used the corresponding age groups of the American National Health Survey of chronic disease in the United States [quoted by Joslin (1)]. This abstraction of a portion of the American survey seems justified, since the over-all rate of 3.7 known diabetics per thousand of the population found by that survey agrees fairly well with our own

TABLE 4. AGE AND SEX INCIDENCE OF DIABETIC CASES

Age at onset	Diabetics seen at hospital		Population of Glasgow (1931 census)		Diabetic patients seen per 100,000 of Glasgow population	
	Males	Females	Males	Females	Males	Females
yrs.						
0-10	5	3	113,268	112,066	4	3
11-15	11	12	45,696	45,690	24	26
16-20	25	24	48,248	52,539	52	46
21-25	21	10	46,213	51,505	45	19
26-30	28	33	43,012	47,485	65	69
31-35	36	35	36,457	43,232	99	81
36-40	25	44	34,466	39,701	73	111
41-45	35	80	31,454	35,611	111	225
46-50	29	130	30,641	33,169	95	392
51-55	55	174	28,317	29,342	194	593
56-60	49	137	25,106	24,590	195	557
61-65	40	120	18,323	18,425	218	651
66-70	18	51	11,978	13,823	150	369
71-75	8	24	6,999	9,531	114	252
76-80	4	2	3,047	4,700	131	43
81-85	1	1	980	1,854	102	54
Unclassified	12	27	—	—	—	—
Up to 40	151*	161*	367,360	392,218	41.1	41.0
41 up	239*	719*	156,845	171,045	152	420
Unclassified	12	27	—	—	—	—
Total	402	907	—	—	—	—

* $\chi^2 = 60.82$; $P = < 0.01$ (highly significant).

estimate³ of 3.9 per thousand in Scotland. Table 3 indicates that diabetes was more frequent in the male Scottish recruits than in the corresponding age groups of the American survey; on the other hand female recruits showed a lower incidence than was found by the American survey. This low incidence in female recruits is not likely to be explained by the infrequency of married women among them, for it will later be shown that marriage is not a factor in the incidence of diabetes until after the age of 35.

³ The total number of diabetics in Scotland was estimated by multiplying the true annual number of deaths from diabetes (Table 1) by the average duration of the disease computed for our clinic cases according to the expectations of life given by Joslin (1), *i.e.*, 1163 deaths by an average duration of 16.7 years, giving a total of 19,420 diabetics alive in Scotland, or 3.9 per thousand of the 1938 population.

It is therefore disturbing to find that Scottish recruits show a totally different incidence of diabetes in the sexes at an age when clinical experience shows a similar frequency in men and women (Table 4).

Numerically, young adult diabetics are an unimportant group. In middle life, diabetes is much more common but we have unfortunately no data to offer on the frequency of undiagnosed diabetes at this age. In view of this we must insist that our data are valid only in respect of the known diabetic population of Scotland.

c) *Control population.* For several of our observations a control population was used. This consisted of a random sample of hospital visitors, who were therefore of approximately the same social class as our diabetics.

TABLE 5. DEATH RATE FROM DIABETES ACCORDING TO AGE AT DEATH (CALCULATED FROM THE ANNUAL RETURNS OF THE REGISTRAR-GENERAL FOR SCOTLAND)

Age at death Yrs.	Average Annual Death Rate* for 1931-1940		
	Males	Females	Female/male ratio
0-	8.1	8.3	1.02:1
15-	30	24	0.80:1
35-	72	102	1.42:1
55 upwards	482	955	1.98:1

* Number of deaths per million of the population of similar age and sex.

Known diabetics were excluded from this group. In the section on heredity, nondiabetic patients were included in the control group.

SEX AND AGE INCIDENCE OF DIABETES

More women than men came to our clinic, the ratio being 2.26 females to every one male. This is in agreement with recent statistics from the United States and from most European countries (1).

The onset of symptoms was taken to represent the onset of the disease. In most of our cases diabetes started after the age of 40 (Table 4), the average age at onset being 45.1 years for men and 50.6 years for women. The tendency for diabetes to occur in middle life is actually greater than these figures would suggest, for the population after the age of 40 dwindles rapidly. In order to correct for this we have considered also the number of diabetics seen by us per 100,000 of the population of Glasgow of similar age and sex (Table 4). These adjusted figures (which must not be taken as absolute rates for diabetes in the population of Glasgow) show that up to the age of 40 both sexes are equally subject to diabetes; after this age

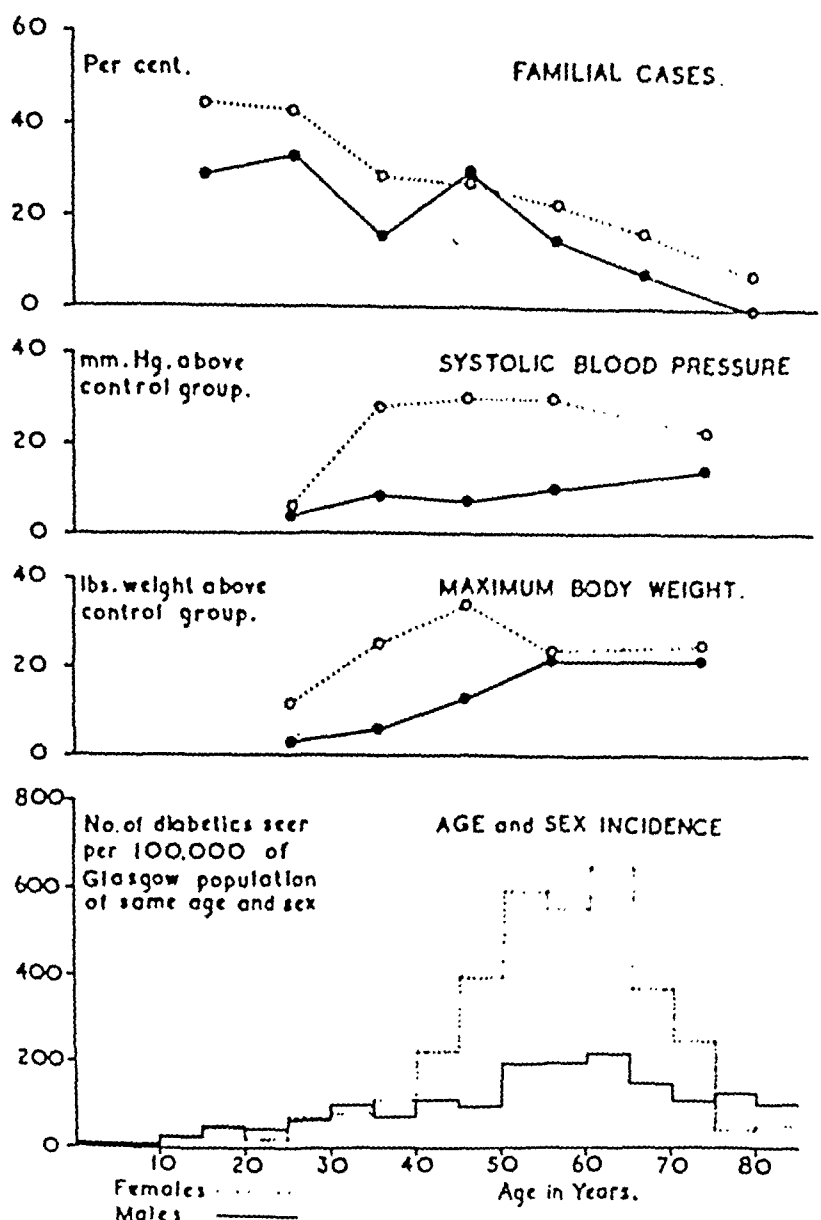


FIG. 2. Age and sex incidence, overweight, hypertension and frequency of familial cases among Scottish diabetics.

its frequency in women increases rapidly while in men the increase does not become obvious until the age of 50 and is much less dramatic (Table 4 and Fig. 2). This finding is supported by the number of deaths from diabetes per million of the Scottish population at different ages (Table 5). During 1931-40, deaths from diabetes in Scotland were slightly more frequent in males up to the age of 34, but after this age more women than men died of diabetes.

THE INCIDENCE OF DIABETES IN MARRIED AND UNMARRIED WOMEN

When the proportion of married and unmarried women at our clinic was compared with the proportion present at similar ages in the general popu-

lation of Glasgow, it was found that married women formed an unduly high proportion of the diabetics from the age of 35 onwards (Table 6). When our cases of diabetes were considered per 100,000 of the population of Glasgow (Fig. 3), the disease was found to occur with the same fre-

TABLE 6. PROPORTIONS OF MARRIED AND UNMARRIED WOMEN DIABETES SEEN AT OUR CLINIC, COMPARED WITH THE PROPORTIONS OF MARRIED AND UNMARRIED WOMEN IN THE GENERAL POPULATION OF GLASGOW (1931 CENSUS)

Age group*	No. in group	Married	Unmarried	χ^2 test
25-34 years:		56	57	0.54†
Diabetics	59	64	36	
General population	92,610	60	40	
35-44 years:		85	15	3.53‡
Diabetics	109	78	22	
General population	76,391	78	22	
45 years upwards:				47.90†
Diabetics	653	93.0	7.0	
General population	143,256	82.7	17.3	

* For diabetics, age and marital status at time of onset of diabetes.

† Statistically significant difference between groups.

‡ No statistically significant difference.

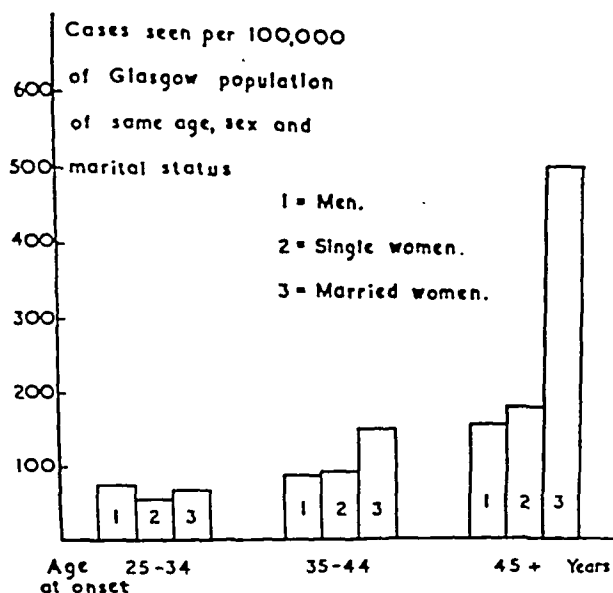


FIG. 3. The relative frequency of diabetes in men, single women and married women.

quency in men and in unmarried women, but to be rather more common in married women between 35 and 44 years of age, and much more common in married women after this age. Thus the higher incidence of diabetes in women as compared with men is due to a factor or factors associated with marriage. This conclusion is supported by recent mortality statistics from North America, where the death rate from diabetes per 100,000 of the population is much the same for men and unmarried women, but is

TABLE 7. DIABETIC SUBJECTS AND HOSPITAL VISITORS DISTRIBUTED ACCORDING TO SIZE OF FAMILY. DATA FROM MARRIED WOMEN 45 YEARS OF AGE OR OLDER AT TIME OF ONSET OF DIABETES OR, IN THE CASE OF CONTROL, POPULATION, AT TIME OF QUESTIONING

	Diabetics		Controls	
	No. in group	Percentage of all subjects	No. in group	Percentage of all subjects
Total number of married women questioned	328	—	147	—
Those with:				
0-3 children	121	37	87	59
4-6 children	101	31	38	26
7 or more children	106	32	22	15
χ^2 test		23.71*		

* The distribution of the diabetic subjects is significantly different from that of the control subjects.

considerably greater for married women (1, 13, 14). A difference in the death rates for married and unmarried women has also been noted in England and Wales (15).

The high incidence of diabetes in married women appears to be related, at least in part, to previous childbearing. When women who developed diabetes after the age of 44 were compared with hospital visitors of similar age (Table 7), it was found that a much higher proportion of the diabetic women had had large families. This finding indicates that women with large families are specially liable to develop diabetes. Marriage and childbearing did not, however, affect the mean age at onset of diabetes in the group (Table 8), nor did they seem to influence the severity of the disease: the proportion of women diabetics who showed ketonuria (more than a trace by Rothera's test at any one visit to the clinic) and the proportion who

TABLE 8. AGE AT ONSET AND SEVERITY OF DIABETES IN RELATION TO MARRIAGE AND PREVIOUS CHILDREARING. DATA FROM WOMEN 45 YEARS OR OLDER AT TIME OF ONSET OF DIABETES

Group of Women	No. in Group	Mean Age at Onset	Severity of Diabetes	
			Patients showing ketonuria	Patients taking insulin
Unmarried	32	57.8 \pm 1.46	16	50
Married:				
No children	37	56.9 \pm 1.31	11	38
1-3 children	84	54.4 \pm 0.60	8	40
4-6 children	101	55.8 \pm 0.61	11	38
7 or more children	106	56.6 \pm 0.50	6	48
χ^2 test	—	—	3.72*	3.56*

* The percentages do not differ significantly.

required insulin for stabilization did not change significantly with marital status or with the number of previous pregnancies (Table 8).

TABLE 9. HEIGHTS (WITH SHOES) OF AN ADULT DIABETIC POPULATION AND AN ADULT CONTROL POPULATION

Group	Diabetics		Controls		Difference	"t" test
	No. in group	Mean height	No. in group	Mean height		
		in.		in.	in.	
21-40 years:†						
Males	17	67.91 \pm 0.69	43	66.94 \pm 0.40	+0.97	1.22*
Females	11	64.16 \pm 0.81	98	63.14 \pm 0.27	+1.02	1.20*
41 years up:†						
Males	28	66.58 \pm 0.52	86	66.21 \pm 0.37	+0.37	0.52*
Females	68	61.82 \pm 0.35	112	62.73 \pm 0.23	-0.91	2.26§

* Difference not statistically significant.

† Age at onset for diabetics; age at time of examination for control population.

§ Difference statistically significant.

HEIGHTS AND WEIGHTS OF ADULT DIABETICS

Heights. The heights of some of our adult diabetics were known and were compared with the heights of visitors to the hospital. Table 9 shows

TABLE 10. BODY WEIGHTS OF A DIABETIC AND A NORMAL ADULT POPULATION. WEIGHTS RECORDED ARE SUBJECT'S ESTIMATED HEAVIEST AND INCLUDE CLOTHING

Age Group*	Diabetics		Controls		Difference	"t" test
	No. in group	Mean body weight	No. in group	Mean body weight		
years		lbs.		lbs.	lbs.	
<i>Males:</i>						
21-30	30	148.1 \pm 4.16	16	145.7 \pm 4.90	+ 2.4	0.34†
31-40	40	156.4 \pm 3.79	42	150.4 \pm 3.08	+ 6.0	1.21†
41-50	47	172.2 \pm 4.03	46	159.4 \pm 4.13	+12.8	2.19§
51-60	64	177.5 \pm 3.94	35	156.7 \pm 3.99	+20.8	3.39§
61 upwards	32	188.3 \pm 5.63	32	166.8 \pm 5.45	+21.5	2.69§
<i>Females:</i>						
21-30	23	128.1 \pm 4.15	32	116.7 \pm 2.53	+11.4	2.42§
31-40	49	161.7 \pm 4.71	67	136.1 \pm 2.71	+25.6	4.95§
41-50	159	180.4 \pm 3.04	60	146.6 \pm 3.64	+33.8	6.20§
51-60	206	178.7 \pm 2.17	41	155.4 \pm 3.25	+23.3	4.57§
61 upwards	96	177.6 \pm 3.49	33	152.2 \pm 4.01	+25.4	3.93§

* Age at onset for diabetics; age at time of examination for control population.

† Not statistically significant.

§ Difference statistically significant.

that the diabetics were not significantly taller than the control population,⁴ a finding which is in agreement with the observations of Joslin, Dublin and Marks (16) and of Blotner, Hyde and Kingsley (2) for adult diabetics. In contrast, diabetes in childhood tends to occur in tall children (White, in Joslin (1)). Our juvenile cases are too few to make our data worth quoting.

Weights. Diabetic patients and control subjects were asked to state what their maximum weights had been and it was found that women diabetics were significantly heavier than the control population from the age of 20

⁴ Women acquiring diabetes after the age of 40 were significantly shorter than the corresponding control group (Table 9). This is due to the large number of multiparous women found among diabetics of this age. In both the diabetic and the control group we noted that women with large families tended to be of small stature, presumably because they originate mainly from the poorer classes of the community.

onwards, whereas the male diabetics were heavier from the age of 40 onwards (Table 10 and Fig. 2). Many authors have remarked that adult diabetics frequently give a history of previous obesity, and Joslin, Dublin and Marks (17) have demonstrated convincingly that a high incidence of diabetes occurs in occupations which favour obesity. Moreover, it has been shown that reduction in body weight may restore the sugar tolerance of obese diabetics (18), especially if the patients are treated early (19). These observations suggest that obesity not only precedes diabetes, but also plays a part in the production and maintenance of the diabetic state.

Does obesity account for the greater susceptibility of married women to diabetes? In Table 11 are shown our data for women diabetics and women

TABLE 11. MAXIMUM BODY WEIGHT IN RELATION TO MARRIAGE AND PREVIOUS CHILD-BEARING. DATA FROM WOMEN 45 YEARS OR OLDER AT TIME OF ONSET OF DIABETES, OR, IN THE CASE OF THE CONTROL POPULATION OF HOSPITAL VISITORS, AT TIME OF QUESTIONING

Group of Women	No. in Group		Maximum Body Weight Lbs.			
	Diabetics	Controls	Diabetics	Controls	Difference	"t" test
Unmarried	32	26	153.8 \pm 5.1	136.5 \pm 4.0	+17.3	2.54*
Married:						
No children	35	13	166.0 \pm 5.1	144.6 \pm 7.2	+21.4	2.26*
1-3 children	82	73	172.3 \pm 4.0	146.6 \pm 3.0	+25.7	5.03*
4-6 children	106	38	185.8 \pm 3.8	159.1 \pm 4.1	+26.8	3.93*
7 or more children	97	21	186.5 \pm 3.1	159.1 \pm 4.6	+27.4	3.98*

* Difference statistically significant.

controls arranged according to marital status and number of children. In both the diabetic and the control group, married women were heavier than single women, and women with large families reached the greatest average weight. If we accept the evidence that obesity can be a factor in the production of diabetes, the greater frequency of obesity in married women provides one likely explanation for their higher susceptibility to diabetes. This subject has also been examined by Joslin (20). He studied women with onset of diabetes after the age of 45 and found that married diabetics had an average weight of 181 pounds, whereas unmarried diabetic women had an average weight of 161 pounds. He did not, however, find any difference between the average weights of childless and fertile married women with diabetes, but it should be noted that Joslin's clinic may not be a representative sample of diabetics (Table 2).

BLOOD PRESSURE IN DIABETES

It was our intention to record blood pressure readings as soon after the commencement of symptoms of diabetes as possible. For this reason, we include in Table 12 only the data obtained from patients who had had symptoms for less than one year before the date of examination. It was subsequently found that the blood pressure readings taken in all new cases seen at the clinic gave results almost identical with those shown in Table

TABLE 12. BLOOD PRESSURES OF A DIABETIC AND A NORMAL ADULT POPULATION (INCLUDES ONLY CASES SEEN WITHIN ONE YEAR OF THE ONSET OF SYMPTOMS)

Age group*	Diabetics		Controls		Difference	"t" test
	No. in group	Mean systolic blood pressure	No. in group	Mean systolic blood pressure		
years		mm. Hg		mm. Hg	mm. Hg	
<i>Males:</i>						
21-30	19	126.8 \pm 4.31	11	122.7 \pm 4.47	+ 4.1	0.60†
31-40	16	136.3 \pm 6.40	28	128.2 \pm 2.58	+ 8.1	1.34†
41-50	23	136.1 \pm 4.36	35	129.4 \pm 3.13	+ 6.7	1.26†
51-60	42	153.6 \pm 3.83	22	143.6 \pm 5.70	+10.0	1.44†
61 upwards	31	165.8 \pm 4.95	16	151.9 \pm 5.81	+13.9	1.69†
<i>Females:</i>						
21-30	8	123.8 \pm 4.66	29	117.6 \pm 2.37	+ 6.2	1.17†
31-40	23	146.1 \pm 6.89	64	117.9 \pm 1.93	+28.2	5.32§
41-50	70	162.3 \pm 3.17	58	132.6 \pm 2.75	+29.7	6.87§
51-60	114	176.3 \pm 2.80	36	146.7 \pm 4.53	+29.6	5.25§
61 upwards	85	181.5 \pm 3.37	27	158.9 \pm 5.72	+22.6	3.29§

* Age at onset for diabetics; age at time of examination for control population.

† Difference not statistically significant.

§ Difference statistically significant.

12 and we have used these more extensive data in all later tables relating to blood pressure.

Each diabetic's blood pressure was taken at the first visit to the clinic; after the patient had been lying on a couch for 15 to 30 minutes, readings were taken with the patient still in the recumbent position. It was impracticable to take the blood pressures of the control group (hospital visitors) under the same conditions; these subjects were examined seated, by the two observers who had taken most of the readings on the diabetics (Table 12 and Fig. 2). In view of the somewhat different conditions under which

the control population had to be examined, it was decided to retake the blood pressure readings of 250 diabetics who had attended the clinic for not more than five years. These readings were taken, with the patients seated, by a single observer who also studied a control group under similar conditions. This second group of data differ from those given in Table 12 in only one respect, *viz.* that the mean pressure of women diabetics between the ages of 40 and 60 years is slightly lower than the figures given in the table. As this does not affect the significance of the data given in the table we shall not give details about this second examination of patients and controls.

Table 12 shows that the mean systolic pressure of the female diabetics is significantly higher than that of the female control group from the age of 30 onwards. Blood pressure also tends to be high in males developing diabetes late in life, but the mean pressure of these cases is not significantly different from that of the control series. Somewhat similar observations were made by Major (21), who compared diabetics (duration of disease unstated) with a control group composed of hospital visitors and hospital staff. He found that the average blood pressure of the diabetics was significantly greater than that of the controls from the age of 50 onwards, and inspection of his data suggests that this was due to hypertension among the women diabetics only. However, Strauss (22) and Brull and Decharneux (23) compared diabetic and nondiabetic hospital cases and found that hypertension was more common in diabetics of both sexes. Blotner, Hyde and Kingsley (2) state that 7 per cent of male diabetic army recruits had hypertension, as compared with 2 per cent among nondiabetic recruits. Although statistical analysis shows this difference in incidence to be significant, it probably can be accounted for by the fact that the recruits with diabetes were older than the nondiabetic recruits. Wollaege (quoted by Wilder (24)) states that the blood pressure of diabetics is not raised, but his control group consisted of patients attending the Mayo Clinic on account of headaches.

Hypertension and obesity. Obesity by itself causes a small increment in the blood pressure of healthy subjects (25, 26) and it has been claimed by Brull and Decharneux (23) that the unusual frequency of hypertension in diabetics is linked with their obesity. In analyzing their data, however, Brull and Decharneux did not consider male and female cases separately and it is possible that the apparent association between obesity and hypertension shown by their data was due to the fact that both of these conditions are specially frequent in diabetic women. Our own observations on this point are confined to women diabetics over 40 years of age at the time of examination. Using standard tables of weight, cases were arranged according to degree of obesity at the time of examination (Table 13). Two

TABLE 13. THE BLOOD PRESSURE OF WOMEN DIABETICS CONSIDERED IN RELATION TO BODY WEIGHT. NO ACCOUNT HAS BEEN TAKEN OF THE DURATION OF THE DISEASE

Group	Age*					
	41 to 50 years		51 to 60 years		61 years upwards	
	No. in group	Mean systolic blood pressure	No. in group	Mean systolic blood pressure	No. in group	Mean systolic blood pressure
		mm. Hg		mm. Hg		mm. Hg
Control group	58	132.6 \pm 2.75	36	146.7 \pm 4.53	27	158.9 \pm 5.72
Diabetic group:						
Underweight*	13	143.8 \pm 6.18	56	165.6 \pm 3.81	55	191.8 \pm 4.13
Up to 24% overweight*	21	174.5 \pm 6.03	80	183.2 \pm 3.51	33	182.2 \pm 4.80
25% or more overweight*	27	171.9 \pm 5.08	53	181.6 \pm 3.64	16	186.2 \pm 7.73

* At time of examination.

features of the data suggest that obesity does not play a large part in the hypertension of middle-aged diabetic women: a) patients who were underweight had a higher average blood pressure than the control group, especially in the later age groups; b) patients who were more than 25 per cent overweight had the same average blood pressure as patients who were less than 25 per cent overweight.

Hypertension and childbearing. We were unable to relate the hypertension of women diabetics to previous childbearing. When diabetic and control subjects were arranged according to size of family, it was found that those without children had the same average pressure as those with large families (Table 14). Our data also suggest that hypertension is not one of the factors

TABLE 14. BLOOD PRESSURE IN RELATION TO MARRIAGE AND PREVIOUS CHILDBEARING. DATA FROM WOMEN 45 YEARS OR OLDER AT TIME OF ONSET OF DIABETES OR, IN THE CASE OF THE CONTROL POPULATION, AT TIME OF EXAMINATION. BLOOD PRESSURE READINGS TAKEN AT PATIENT'S FIRST VISIT TO THE CLINIC

Group of Women	Diabetics		Controls	
	No. in group	Mean systolic blood pressure	No. in group	Mean systolic blood pressure
		mm. Hg		mm. Hg
Unmarried	22	168.2 \pm 5.1	13	145.4 \pm 9.4
Married:				
No children	34	177.4 \pm 5.5	28	143.2 \pm 4.3
1-3 children	84	171.0 \pm 2.9		
4-6 children	100	174.3 \pm 2.8	28	142.9 \pm 4.7
7 or more children	106	175.1 \pm 3.0		

There are no significant differences between the different groups of diabetics or between the different groups of control subjects.

causing diabetes to occur more frequently in women than in men. The specially high incidence of diabetes in middle-aged women is confined to married women, and if this were partly due to hypertension, one would expect to find a larger proportion of hypertensive cases and therefore a higher average blood pressure among married than among unmarried diabetic women. Table 14 shows that the mean blood pressure is the same for married and unmarried diabetic women.

THE HEREDITARY FACTOR IN DIABETES

The terms "familial" and "nonfamilial," as used in this paper, refer to whether or not a family history of diabetes was obtainable. Of 923 diabetics questioned, 23.2 per cent were found to be familial cases (Table 15). On re-

TABLE 15. INCIDENCE OF FAMILIAL CASES OF DIABETES

Age at onset of diabetes	Total number of cases	Those known to have diabetic relatives		χ^2 test
		Total number	Per cent of cases	
<i>Males:</i>				
Up to 40 years	108	26	24	} 2.29†
41 years upwards	168	28	17	
<i>Females:</i>				
Up to 40 years	104	37	36	} 7.83*
41 years upwards	543	123	23	

* The percentages are significantly different.

† The percentages do not differ significantly.

questioning 213 of them after they had been diabetic for more than five years, the figure obtained was 24.9 per cent, essentially the same proportion as previously ($\chi^2 = 0.28$; $P = 0.7 - 0.5$). These figures agree with most other estimates of the frequency of known familial cases among adult diabetics. A group of 2043 nondiabetic patients and hospital visitors was also investigated; only 5.3 per cent of these had family histories of diabetes. This is significantly less than the incidence among the diabetics ($\chi^2 = 208.8$; $P = < 0.01$).

Thus a history of diabetes in the family can be elicited in only some cases. The proportion is highest when the disease begins early in life and is lowest when it starts late in life (Fig. 2). This observation is supported by the findings of Cammidge (27) and of Rudy and Keeler (28), but not by those of Joslin, Dublin and Marks (29). We shall now consider whether

this decline in the proportion of familial cases as age at onset advances can be related to any of the clinical features of diabetes.

a) *Severity*. It is generally conceded that diabetes in the young tends to be more severe than diabetes starting later in life. The decline in severity

TABLE 16. BODY WEIGHT PRIOR TO THE ONSET OF DIABETES, BLOOD PRESSURE AT THE TIME OF THE FIRST VISIT TO THE CLINIC, AND SEVERITY OF THE DISEASE IN DIABETICS WITH AND WITHOUT A FAMILY HISTORY OF DIABETES

Age at onset	Body weight		Blood pressure		Severity of diabetes			
	No. in group	Mean weight	No. in group	Mean systolic blood pressure	No. in group	Mean daily carbohydrate intake	Patients showing ketonuria	Patients taking insulin
		lbs.		mm. Hg.		Gm.	%	%
<i>16-40 years:</i>								
<i>Males:</i>								
Familial	18	146 \pm 5.7	20	132 \pm 2.7	26	142	42	81
Nonfamilial	60	151 \pm 3.4	59	128 \pm 2.3	78	144	39	74
<i>Females:</i>								
Familial	28	145 \pm 7.8	33	141 \pm 5.4	35	119	37	89
Nonfamilial	56	148 \pm 3.0	66	142 \pm 3.5	72	111	44	75
<i>41 years upwards:</i>								
<i>Males:</i>								
Familial	27	176 \pm 6.4	29	159 \pm 6.0	28	130	21	57
Nonfamilial	116	179 \pm 3.0	141	153 \pm 2.2	144	133	18	56
<i>Females:</i>								
Familial	95	183 \pm 4.5	129	171 \pm 2.7	124	112	17	47
Nonfamilial	366	178 \pm 1.7	459	174 \pm 1.4	427	110	14	47

(The data for familial and nonfamilial diabetics of similar age and sex do not differ significantly in any of the above groups.)

coincides with the fall in the frequency of familial cases, and it might be suspected that the mild cases of diabetes seen in elderly people were "non-familial." However, when patients of comparable age were divided into familial and nonfamilial cases, it was found that the same proportion in each group required insulin for stabilization and that the same proportion in each group had ketonuria (Table 16). Thus there is no reason to suspect that nonfamilial diabetics are milder than cases known to have a family history of the disease.

b) *Obesity and hypertension*. Obesity is common among adult diabetics and hypertension is frequent in middle-aged diabetic women (Fig. 2), and both might be considered as possible nonhereditary causes of diabetes. The relationship of obesity and hypertension to the hereditary factor was investigated in two ways. First, the diabetics were arranged into familial and nonfamilial groups, and it was found that both groups had the same aver-

age blood pressure and the same average weight (Table 16). Secondly, middle-aged diabetic women were grouped according to degree of obesity and according to blood pressure (Table 17); the percentage of familial cases was found to be essentially similar in the various groups. There is accordingly no reason to suspect that nonfamilial cases of diabetes occur with disproportionate frequency among obese or hypertensive subjects.

TABLE 17. THE FREQUENCY OF FAMILIAL CASES OF DIABETES CONSIDERED IN RELATION TO BODY WEIGHT AND IN RELATION TO BLOOD PRESSURE (DATA FROM WOMEN OVER 40 YEARS OF AGE AT TIME OF ONSET OF DIABETES)

Group of women	No. in group	Mean age at onset	Those with diabetic relatives
		yrs.	%
<i>Maximum Body Weight:</i>			
Underweight and up to 24% overweight	164	57.0	26.2
25% to 49% overweight	113	54.5	22.1
50% or more overweight	66	53.0	28.8
χ^2 test	—	—	1.10*
<i>Systolic Blood Pressure:</i>			
Up to 144 mm. Hg	95	52.8	22.1
145 to 194 mm. Hg	318	55.8	23.6
195 mm. Hg upwards	108	58.2	20.4
χ^2 test	—	—	0.50*

* The percentages do not differ significantly. The patients were also classified into 10-year age groups and then arranged according to body weight and according to blood pressure. The values of χ^2 so obtained did not reach significance in any one decade or in the aggregate of decades.

c) *Previous childbearing.* Family histories were investigated in women developing diabetes after the age of 44 years and in a control series of non-diabetic patients of similar age; both groups were arranged according to marital status and number of children (Table 18). In the diabetic group, a family history of the disease was less frequently obtained from women with large families than from those with small families. This is not due to differences in the mean ages of the various groups (Table 8): nor is it due to greater ignorance of the family history among women with large families, for in the control group family histories of diabetes were just as frequently obtained from women with large families as from the rest of the group (Table 18). It has therefore been concluded that some factor associated

with previous childbearing increases the frequency of nonfamilial cases of diabetes, resulting in a smaller proportion of familial cases among women with large families. Although such women have a greater maximum body weight than women with small families (Table 11), obesity is not the reason for the larger proportion of nonfamilial cases of diabetes among fertile women, for previous childbearing was found to affect the proportion of

TABLE 18. THE FAMILIAL FACTOR IN RELATION TO MARRIAGE AND PREVIOUS CHILD-BEARING (DATA FROM WOMEN 45 YEARS OF AGE OR OLDER AT TIME OF ONSET OF DIABETES OR, IN THE CASE OF NONDIABETIC PATIENTS, AT TIME OF QUESTIONING)

Group of women	Diabetic patients		Nondiabetic patients	
	No. in group	Those with diabetic relatives	No. in group	Those with diabetic relatives
Unmarried	32	% 34.4	54	% 9.0
Married:				
No children	37	32.4	57	
1-3 children	84	27.4	200	8.5
4-6 children	101	21.8	131	8.4
7 or more children	106	17.9	88	10.2
χ^2 test	6.24*		—	

* Although the value of χ^2 for the series is not significant, there is a significant difference between the frequency of familial cases among all childless women (married as well as single) and the frequency among women with 7 or more children ($\chi^2 = 5.44$; $P = 0.02 - 0.01$).

familial cases among women having the same degree of obesity (Table 19). Thus obesity is not the only factor which increases the susceptibility of childbearing women to diabetes.

Sex linkage. Penrose and Watson (30) examined the relative frequency of diabetes in brothers and sisters and found that the disease had a definite tendency to recur in the same sex when more than one member of the sibship was affected. They concluded that some factor in the inheritance of diabetes was carried by the chromosomes determining sex. Among the families of our own cases, there were 14 pairs of diabetic brothers, 54 diabetic brother-sister pairs and 100 pairs of diabetic sisters. These data show a suggestive though not significant excess of like-sexed siblings ($\chi^2 = 2.79$; $P = 0.10 - 0.05$) which might be interpreted as supporting a sex-linked in-

herited factor in diabetes. The explanation of this excess of like-sexed siblings is, however, more likely to be provided by social rather than by genetic factors. During 1930-32, deaths occurring in England and Wales were classified according to social status (15) and in the case of diabetes it was found that the highest death rate in males was in the upper classes, whereas the highest death rate in females was in the lower classes. This

TABLE 19. FREQUENCY OF POSITIVE FAMILY HISTORIES RELATED TO SIZE OF FAMILY AMONG DIABETIC WOMEN OF VARIOUS WEIGHTS

Group I: Single women, and married women with up to 2 children.

Group II: Married women with 6 or more children.

Data from women 45 years of age or older at time of onset of diabetes

Group of women	No. in group	Mean age at onset	Those known to have diabetic relatives	χ^2 test
		yrs.	%	
Underweight and up to 19% overweight:				
Group I	54	56.7	30	0.39†
Group II	34	60.6	24	
20 to 39% overweight:				
Group I	17	57.9	35	3.99*
Group II	46	56.2	13	
40% or more overweight:				
Group I	25	50.8	44	7.57*
Group II	38	55.9	13	

* The frequency of familial cases is significantly different in the two groups.

† The frequency of familial cases does not differ significantly in the two groups.

suggests that the sex incidence of diabetes is modified by social status, namely that men form a higher proportion of cases in the upper classes than they do in the lower classes. Since the members of a family tend to remain within the same social class, this means that the occurrence of diabetes in two brothers is favored in the upper classes and the occurrence of diabetes in two sisters in the lower classes. In a group of diabetics representative of all social classes one will therefore encounter pairs of diabetic brothers and pairs of diabetic sisters out of proportion to the number of diabetic brother-sister pairs found. This seems an adequate explanation for the small excess of like-sexed siblings with diabetes which our clinic provides.

Heredity and age at onset. When diabetes occurs in more than one member of the same sibship, it often starts in each at about the same age (30). Thus, on investigating 32 sib-pairs, we found a highly significant correlation coefficient (+0.668) for age at onset. Patients are, however, commonly questioned within a few years of the onset of the disease; in the case of young diabetics, this means that siblings developing diabetes late in life are omitted. The data are therefore biased in favor of an association between ages at onset. In an attempt to eliminate this fallacy, we considered

TABLE 20. AGE AT ONSET OF DIABETES IN PAIRS OF SIBLINGS

(Only clinic cases with onset after the age of 49 years were investigated. In this table the age at onset of diabetes in their siblings (group A) is compared with the age of onset among familial cases in general (group B))

Age at onset Yrs.	Males		Females	
	Group A	Group B	Group A	Group B
	No. of cases	No. of cases	No. of cases	No. of cases
0-20	1	7	1	8
21-30	5	11	1	12
31-40	1	8	3	17
41-50	1	14	20	47
51-60	6	11	26	53
61 upwards	2	3	1	23
Up to 40 years	7	26	5	37
41 years upwards	9	28	47	123
χ^2 test (one degree of freedom)	0.10†		4.51*	

* The two groups differ significantly in age distribution.

† The two groups do not differ significantly in age distribution.

only the siblings of cases who developed the disease after the age of 49 years (Table 20). If the age at onset in siblings is related, then this group of elderly diabetics should rarely provide siblings who developed diabetes when young. Our data show (Table 20) that diabetes starting in brothers of these elderly patients conformed to the general age distribution of the disease in male familial cases, nearly half having had symptoms before the age of 41 years. On the other hand, sisters of these elderly patients provided fewer young diabetics than would be expected on a random distribution. This finding may not, however, be evidence of genetic influence over

the age at which diabetes starts. Other factors may influence both members of a sib-pair. Thus, previous childbearing appears to increase the susceptibility of women to diabetes after they have reached middle age (Table 7). In the lower classes, families are often large and sisters in this social grade might be expected on this account to have a special liability to develop diabetes in middle life. The elucidation of genetic influences on the age at which diabetes starts must accordingly await more comprehensive methods for analyzing the data.

ASSOCIATION OF DIABETES WITH OTHER ENDOCRINE DISEASES

About 2 to 3 per cent of patients with hyperthyroidism also suffer from diabetes (1, 31). Since the thyrotoxicosis occurs mainly in young adults (32) this would appear to be a much higher frequency of diabetes than would be expected by chance (*cf.* Table 3). It must therefore be concluded that the two conditions are related.

Of our 1309 diabetics, 13 (4 men and 9 women) had hyperthyroidism; 3 of these cases were diagnosed solely on clinical grounds but the remaining 10 had sufficiently detailed records to assure us that they conformed to the standards laid down by Joslin and Lahey (33) for the diagnosis of the two diseases in combination. This incidence of hyperthyroidism in diabetes (1.0 per cent) is of the same order as that recorded by most other authors (1, 33, 34, 35), though some estimates are slightly higher (31, 36, 37). According to Joslin (1) the thyrotoxicosis starts either before or at the same time as the diabetes in the majority of cases. If thyrotoxicosis was the only cause of diabetes in such cases, one might expect that the combination would be most common at the age when thyrotoxicosis is most frequent and that a family history of diabetes would be obtained as infrequently as from nondiabetic subjects. The data of Wallace (32) indicate that in Scotland the average age at onset of thyrotoxicosis alone is 30.5 years. Our clinical data show that the average age at which diabetes starts is 45.1 years for men and 50.6 years for women. It is therefore interesting to find that the average age at which diabetes started in our thyrotoxic cases of diabetes was 42 years for males and 43 years for females. Thus the age incidence of diabetes associated with hyperthyroidism is not notably different from that of diabetes alone, a finding which conforms with the observations of John (34) and of Joslin (1). Like Joslin and Lahey (33) and Foster and Lowrie (31), we found a family history of diabetes to be as common among the thyrotoxic cases as among other diabetics, but our cases are too few to carry much weight. The evidence is thus against thyrotoxicosis being the sole cause of diabetes in subjects suffering from both conditions.

Frank pituitary disease was seldom seen at our clinic. Two patients with acromegaly came for treatment of their diabetes and in one case of diabetes

pituitary basophilism was considered to be present. On the other hand, diabetes is well known to be a common sequel to acromegaly. Thus Coggeshall and Root (38) found 29 diabetics among 156 cases of acromegaly seen in Massachusetts. This is of course much too high an incidence of diabetes to occur by chance. A family history of diabetes was obtained from 21 per cent of the acromegalies with diabetes (*i.e.*, about as commonly as at Joslin's diabetic clinic in Massachusetts (29)) and from only 2 per cent of the acromegalies without diabetes. This suggests that, as in the case of thyrotoxicosis, acromegaly is not the only factor involved in the production of diabetes, when the two diseases occur together.

MISCELLANEOUS FACTORS IN THE ONSET OF DIABETES

The influence of infections on the onset of diabetes has been frequently discussed (see Marble, in Joslin (1)). Among our own patients, 6.5 per cent

TABLE 21. SEPSIS ASSOCIATED WITH THE ONSET OF DIABETES

Group I: Diabetic patients without sepsis (age at onset of diabetes)

Group II: Cases where sepsis was apparently associated with onset of diabetes (age at onset of diabetes)

Group III: Consecutive nondiabetic patients admitted to the sepsis wards (age on admission)

Group	No. in group	Mean age	Difference	"t" test
		yrs.	yrs.	
<i>Males:</i>				
Group I	364	44.75 ± 0.87	5.75	1.70†
Group II	26	50.50 ± 3.35		
Group III	312	34.49 ± 1.12	16.10	3.98*
<i>Females:</i>				
Group I	826	50.93 ± 0.45	1.22	0.67†
Group II	54	52.11 ± 1.93		
Group III	249	31.55 ± 1.02	20.56	8.69*

* Difference statistically significant.

† Difference not statistically significant.

of the males and 6.0 per cent of the females were first found to be diabetic when suffering from some septic condition, usually a boil or carbuncle. Table 21 shows that nondiabetic patients admitted to the hospital on account of septic conditions were frequently young people, whereas those in whom diabetes was also found to be present were mainly of middle age. This implies that, if infection does play a part in the onset of diabetes, it usually does so at the age when diabetes occurs most commonly and not at the age when sepsis is most frequent. Furthermore, of the 80 diabetics

in whom the onset of diabetes apparently coincided with a septic process, 18 per cent had a family history of diabetes. This figure does not differ significantly from the frequency of familial cases among other diabetics ($\chi^2 = 1.58$; $p = 0.3 - 0.2$).

In view of the recent experimental production of diabetes with alloxan (39), and its possible implications in purine metabolism, it should be added that no case of gout has been encountered at our diabetic clinic. Gout is a rare disease in Scotland, and its rarity appears to extend to the diabetic population.

DISCUSSION

From our investigation of Scottish diabetics and from an examination of the literature we have concluded that certain features (a history of diabetes in the family, obesity, hypertension, large families, thyrotoxicosis, acromegaly and perhaps sepsis) occur in combination with diabetes more frequently than they should by chance. Since these conditions are found early in the course of the disease, we must consider what part they play in the causation of diabetes.

In the first place, there is the hereditary factor. This can be established in only about a quarter of the cases but many authors assume that the other diabetics, though unable to trace a relative with diabetes, are also hereditary cases. Do any of our observations make this standpoint untenable? Our data show (Fig. 2) that, as age at onset increased, family histories of diabetes were less often obtained and this might be taken to indicate a growing number of nonhereditary cases of diabetes as age at onset advances. However, Joslin, Dublin and Marks (29) give several reasons for believing that such a change in the proportion of hereditary cases may be apparent rather than real. We have also considered whether there is any reason for believing that obesity, hypertension, childbearing, thyrotoxicosis, acromegaly and sepsis are nonhereditary causes of diabetes. If any one of them could act as such, one would expect the proportion of cases with a family history of diabetes to be unusually low among diabetics with that condition. The data show that a family history of diabetes occurred as commonly among diabetics suffering from obesity, hypertension, thyrotoxicosis, acromegaly or sepsis, as among diabetics who did not have these conditions. This indicates that, whatever may be the part played by these conditions in the causation of diabetes, they do not act as nonhereditary causes to any important extent. On the other hand, a significantly lower proportion of familial cases was observed among women diabetics with large families. Before accepting this as conclusive evidence that a nonhereditary form of diabetes occurs among fertile women, it is as well to consider whether there may be any alternative explanation. One such ex-

planation is that the inheritance of diabetes occurs through several genetic defects and that childbearing increases the susceptibility of the individual to inherited diabetogenic factors which do not often appear (low penetrance). A definite decision cannot be arrived at without further work, but we shall assume as a working hypothesis that cases of diabetes occurring among childbearing women are all based on an inherited defect.

In the second place, we must consider the part played in the etiology of diabetes by the factors other than heredity. Since none of these factors (with the possible exception of childbearing) appears to be a nonhereditary cause of diabetes, their relationship to diabetes may take one of three forms: i) that of a condition linked genetically to diabetes. Such a condition would be found in association with diabetes but would not increase susceptibility to diabetes. ii) that of a secondary (aggravating) factor which increases the susceptibility of persons predisposed to diabetes by inheritance. iii) that of an early result of diabetes. We shall consider each feature of the diabetic separately. a) Investigations already referred to (17, 18, 19) make it probable that obesity is a factor in the causation of diabetes and not merely genetically linked to it or occurring as an early stage in the course of the disease. b) From the age of 30 onwards, diabetic women were found to have a higher average blood pressure than a control population of hospital visitors (Table 12). It was concluded, however, that hypertension was not a significant factor in the causation of diabetes in this group of women. It is most likely to be an early result of diabetes. c) Previous childbearing can only fall into the category of a cause of diabetes. d) Evidence from animal experiments shows that thyroid administration can provoke diabetes under suitable conditions of pancreatic impairment (40). Thyrotoxicosis is therefore likely to increase susceptibility to diabetes. e) The existence of a diabetogenic factor in the anterior lobe of the pituitary gland is widely accepted and it is reasonable to assume that this gives acromegalic subjects an increased susceptibility to diabetes. f) The relationship of sepsis to the onset of diabetes is difficult to assess. The well-known effect of sepsis in aggravating the severity of established cases of diabetes indicates that its occurrence prior to the onset of diabetes might increase the individual's susceptibility to diabetes.

Considering these various etiologic factors together, we have tentatively divided them as follows:

- A) A hereditary factor or factors, fundamental to virtually all cases.
- B) Factors increasing the susceptibility of persons predisposed to diabetes by the hereditary factor or factors:
 - i) Obesity.
 - ii) One or more factors associated with childbearing.
 - iii) Minor factors (thyrotoxicosis, acromegaly, ?sepsis).

There may well be other factors which increase susceptibility to diabetes. For example, several authors (3 and 41-45) have described a decline in sugar tolerance which occurs in both normal and obese members of the general population with advancing age. The factors responsible for this have apparently not been investigated.

SUMMARY

1. Certain features of a group of 1309 diabetics have been studied. The group was considered to be representative of Scottish diabetics since the sex distribution of cases corresponded to the sex distribution of deaths from diabetes recorded in the official mortality statistics for Scotland. Evidence is presented which justifies the use of Scottish mortality statistics for this purpose. A study of the incidence of diabetes in 413,110 Scottish recruits (male and female) suggested that in the general population the sex distribution of persons with undiagnosed diabetes might not be the same as that of persons known to have the disease.

2. In both sexes the disease started most frequently after the age of 40 years. The sex incidence was equal up to the age of 40; thereafter, female diabetics were much more common than male diabetics.

3. The high frequency of diabetes in women of middle age was confined to married women and appeared to be related, at least in part, to previous childbearing. The age at onset and the severity of the disease in this group of women were apparently uninfluenced by marriage and childbearing.

4. The adult diabetics were no taller than the control group (hospital visitors). In the case of women diabetics the maximum weight was significantly greater than that of the control population from the age of 20 onwards, and in the case of male diabetics from 40 onwards. In both diabetic and control groups, married women were heavier than single women.

5. The mean blood pressure was significantly higher in female diabetics after the age of 30 years than in the corresponding control group. This hypertension could not be adequately explained on the grounds of obesity; it was not related to previous childbearing.

6. Of 923 diabetics questioned, 23.2 per cent gave a family history of diabetes. As age at onset of the disease increased, positive family histories decreased. There was no relationship between the presence of a positive family history and the severity of the disease. The frequency of a positive family history in obese and hypertensive patients did not differ from that of the group of diabetics as a whole. In the group of middle-aged married women, those with the largest families gave the fewest positive family histories. It was considered that there was no proof of partial sex linkage of the hereditary factor, and no convincing evidence that the age at onset is determined by inherited factors.

7. Thyrotoxicosis was present in 1 per cent of the cases. The age at onset in these cases was similar to that in diabetes generally.

8. Sepsis was associated with the onset of diabetes in 6 per cent of cases, but the average age at onset was that for diabetes in general, and a family history of diabetes was obtained in 18 per cent of the cases.

9. After considering these data, it was concluded that the etiologic factors in human diabetes could be tentatively divided into

- a) A hereditary factor or factors, fundamental to almost all cases.
- b) Factors increasing the susceptibility of persons predisposed to diabetes by the hereditary factor:
 - i) Obesity.
 - ii) One or more factors associated with childbearing.
 - iii) Minor factors (thyrotoxicosis, acromegaly, ?sepsis).

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EFFECT OF ROENTGENOTHERAPY ON URINARY 17-KETOSTEROID EXCRETION IN ANKYLOSING SPONDYLARTHRITIS*

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FEW studies of urinary 17-ketosteroid excretion have been made in rheumatic disease. Fraser and co-workers (1) found diminished excretion in a 16-year-old female with rheumatoid arthritis. Chou and Wang (2) reported low values in rheumatic fever. In 7 males with rheumatic disease, Forbes and associates (3) found the average excretion to be 7.5 mg. in 24 hours, with a range of 2.7 to 11.5 mg., and in females an average of 4.5 mg. with a range of 1.2 to 7.8 mg. Low excretion is found in malnutrition, anemia, hypothyroidism, panhypopituitarism, Addison's disease, and chronic debilitating diseases (1, 2, 3, 4, 5), whereas high excretion occurs in carcinoma of the adrenal cortex, hyperplasia of the adrenals, masculinizing ovarian tumors, hirsutism of females and in Cushing's syndrome (1, 6, 7).

Although ankylosing spondylarthritis is a chronic debilitating disease accompanied by malnutrition, anemia, and pronounced muscle wasting in its advanced stage, we have found increased urinary 17-ketosteroid excretion in all of 13 males having this disease (8). Our study of 17-ketosteroid excretion has been extended and the results are reported in this paper. Since roentgenotherapy exerts a pronounced beneficial effect on the course of the disease in these patients (9, 10, 11, 12), giving relief of symptoms, abatement of objective physical findings, and improved well being even in advanced cases, observations were made to determine the effects of roentgenotherapy on 17-ketosteroid excretion during the course of such treatment. The cases studied comprised 31 males and 4 females. The majority were patients in the outpatient clinic of Stanford University Medical School; a few were private patients. Five males and one female were Army personnel provided by Major Potter, all of whom were examined by one

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TABLE I.

Case	Sex	Age (yrs.)	Duration of symptoms, (yrs.)	Status of disease; chief symptoms and nutritional state of patient at time of initial estimation of 17-ketosteroid excretion	Sodium-tion rate, mm./hr., Winthrobe	Joints affected, as shown in roentgenograms	Initial urinary 17-ketosteroids, mg./24 hrs.	Range of 17-ketosteroid values during treatment, mg./24 hrs.	Roentgen therapy
							No. of courses	Total rhodman*	
1. W.P.	M	23	1	Mild. Pain and stiffness of lower back. Little disability.	24	Sacroiliac.	21.5	20-46 (see Fig. 6)	2 2640
2. D.H.	M	28	1	Active, mild. Backache after standing.	5	Sacroiliac.	29.5		
3. G.C.	M	19	3	Active, rapidly progressing. Diminished chest expansion. Nutrition good.	7	Sacroiliac. (ankylosis); lumbar apophyseal and thoracic.	16.5	6-22	2 1800
4. H.J.	M	21	½	Active, mild. Low back pain and stiffness, 2 mos. Moderate muscle spasm.	35	Sacroiliac and lumbar apophyseal.	34.0	19	1 675
5. M.K.	M	26	1	Active, mild. Early morning pain and stiffness. Lumbar flexion limited by muscle spasm.	20	Left sacroiliac, bony.	15.0		3 2700
6. R.C.	M	30	1	Active, rapid progression in 6 mos. Low back pain and stiffness. Nutrition good.	47	Sacroiliac, sclerotic. X-rays 6 mos. before showed little change.	18.5		3
7. L.N.	M	22	1	Active, rapidly progressing. Much lumbar pain and stiffness and muscle spasm.	14	Sacroiliac, blurred.	20.0	16-39.5 (see Fig. 5)	
8. L.K.	M	30	2	Inactive, mild. Stiffness of lower back. Previous gonorrhea.	10	Sacroiliac, sclerotic.	39.0		
9. R.P.	M	25	1½	Active, rapidly progressing. Much pain in lumbar and dorsal spine with limitation of movement by muscle spasm. Febrile.	12	Sacroiliac, bony.	27.0	6-22 (see Fig. 2 and 3)	2 2400
10. E.G.	M	36	3	Active, mild. Pain and stiffness in neck and lower back. Associated hirsutism. Nutrition good.	16	Sacroiliac.	36.0		
11. W.S.	M	40	2	Active, severe. Very stiff back and much pain.	44	Sacroiliac.	24.0	9.2-14	
12. J.C.	M	25	3	Active, mild, rapidly progressing. Scoliosis and rigid lumbar spine. Nutrition good.	30	Right sacroiliac, bony.	34.0	23-37	1 1200
13. C.C.	M	24	3	Active, rapidly progressing. Nutrition good.	32	Sacroiliac, sclerotic.	40.0		
14. W.G.	M	39	4	Stiffness in lower back only. Nutrition good.	40	Sacroiliac and lumbar, ankylosed.	25.5	22-47	3 1085
15. G.E.	M	33	4	Active, severe, rapidly progressing. Complete disability. Stiffness of spine and swelling of peripheral joints.	53	Sacroiliac, moderately sclerotic; lumbar 1-5, calcified ligaments.	26.0	13.2-41.8	1 900
16. V.K.	M	28	4	Active, severe. Much disability. Dorsal kyphosis.	45	Sacroiliac, lumbar, dorsal, and cervical. Entire spine ankylosed.	34.0	12.5-34	3 5150
17. R.K.	M	33	7	Severe pain and stiffness of hips and neck.	20	Sacroiliac and lumbar, calcified ligaments.	30.0	30-42.5	1 2200
18. W.L.	M	30	6	Active, mild. Stiff lumbar and dorsal spine. Nutrition good.	28	Sacroiliac, ankylosed.	19.0	17.5-24.5	2 3680

TABLE 1.—(Continued)

Case	Sex	Age (yrs.)	Duration of symptoms (yrs.)	Status of disease; chief symptoms and nutritional state of patient at time of initial estimation of 17-ketosteroid excretion	Sedimentation rate, mm./hr., Wintrobe	Joints affected, as shown in roentgenograms	Initial urinary 17-ketosteroids, mg./24 hr.	Range of 17-ketosteroid values during treatment, mg./24 hr.	Roentgen therapy
19. C.T.	M	38	10	Active, mild. Patient capable of mild activity. Stiffness of entire spine. Nutrition good.	12	Sacroiliac, lumbar, and dorsal, ankylosed.	61.0		
20. W.H.	M	25	10	Active, severe. Much pain, stiffness of lumbar and dorsal spine and loss of strength. Nutrition poor.	12	Sacroiliac (sclerosis and cystic changes); lumbar apophyseal, bony.	19.0		1
21. B.Y.	M	25	7	Active, mild. Stiff lumbar spine. Nutrition good.	12	Right sacroiliac; all lumbar.	22.5	14-22.5	2
22. F.Y.	M	39	18	Little disability. Nutrition poor.	13	Sacroiliac, ankylosed; right hip.	22.5	22.5-35	3
23. C.B.	M	37	10	Advanced disease. Whole spine stiff. Nutrition good.	32	Sacroiliac, lumbar, and dorsal; calcified ligaments.	13.5		1
24. F.F.	M	35	15	Entire spine stiff. Muscular weakness and fatigue. Nutrition poor.	25	Sacroiliac to cervical, completely ankylosed.	25.0	6.2-25	1
25. W.G.	M	32	13	Bedridden. Stiffness of entire spine. Emaciation and muscular atrophy.	15	Sacroiliac, cervical, dorsal, and lumbar.	10.5	6-22.5 (see Fig. 5)	
26. M.M.	M	30	17	Active, severe. Completely stiff spine. Severe pain.	37	Sacroiliac, lumbar, dorsal, and cervical; calcified ligaments.	23.5		
27. W.P.	M	43	20	Active, mild. Completely stiff spine.	35	Sacroiliac, lumbar, dorsal, and cervical; calcified ligaments.	23.5		
28. B.P.	M	42	10+	Active.	26	Sacroiliac, lumbar, dorsal, and cervical.	27.0	10.5-39	2
29. R.S.	M	41	2(?)	Inactive, slight cervical pain. Entire spine stiff. Nutrition excellent.	8	Sacroiliac, ankylosed; lumbar and dorsal, calcified ligaments.	31.0		
30. W.M.	M	30	7	Inactive. Stiff spine. No symptoms. Previous x-ray therapy.	20	Sacroiliac, lumbar, dorsal, and cervical.	22.5		
31. W.N.	M		3	Active, rapidly progressing.	20	None	20.0		
32. R.H.	F	25	6	Aching and stiffness of lower back.	45	Right sacroiliac, bony.	19.5		
33. E.R.	F	39	14	Active, slowly progressing. Pain and tenderness of dorsal spine.	24	Sacroiliac, ankylosed; lumbar 1-2, calcified ligaments.	10.0		
34. L.B.	F	41	18	Active, mild. Stiff neck and lower back.	30	Sacroiliac and cervical	23.0		
35. M.O.	F	32	7	Some back pain. Nutrition excellent. Infectious type of anemia. Previous x-ray therapy.	19	3-4-5 lumbar apophyseal, ankylosed.	16.0		

* Skin roentgens.

of us (R.D.). Four were Navy personnel whose records and roentgenograms were made available to us by Commander Cuttle.

The occupations of these patients were varied. The ages ranged from 19 to 43 years; duration of symptoms from less than one, to twenty-six years. Two of the patients (cases 30 and 35) had roentgenotherapy prior to our examination of them. The disease showed all grades of activity; in three patients it appeared to be relatively inactive; in the remainder, including

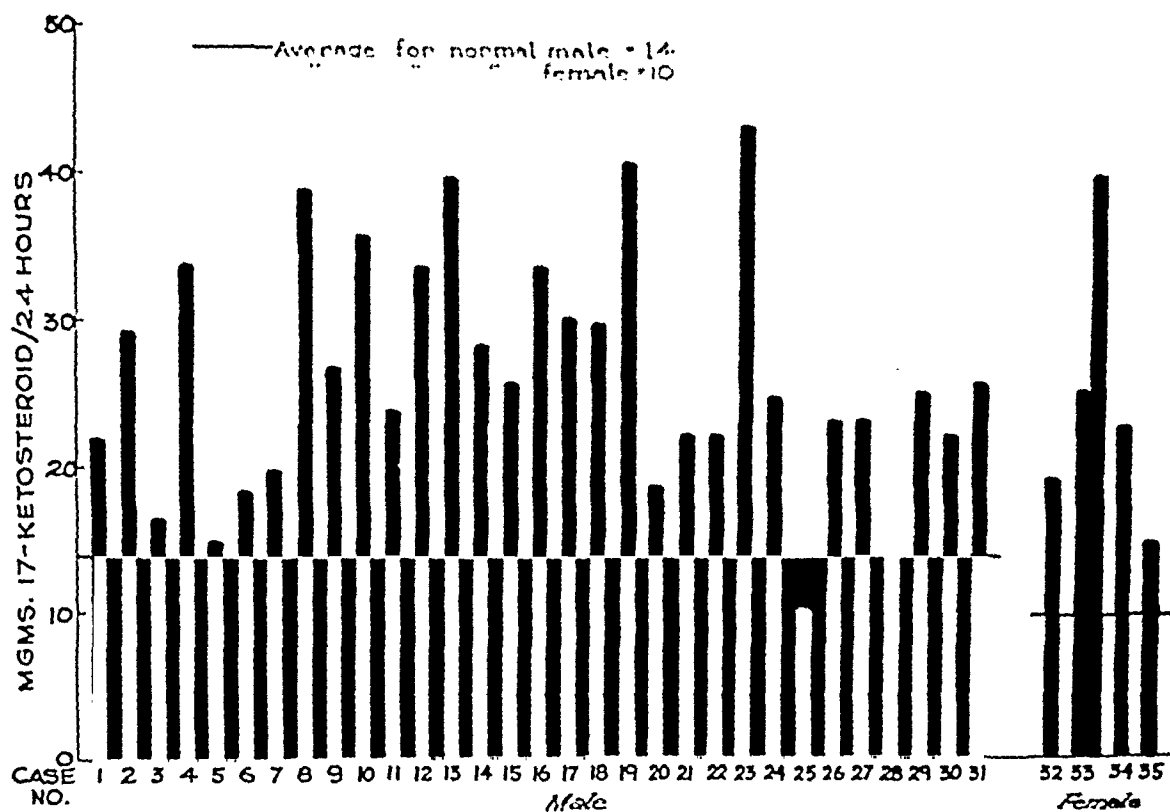


FIG. 1. Urinary excretion of 17-ketosteroids (mg.) in 24 hours in males and females with ankylosing spondylarthritis.

those with histories dating back many years, there was objective evidence of active disease. The nutritional state varied from excellent to poor. The clinical status of the individual patients is given in Table 1.

Roentgenograms were made of the sacroiliac joints and the entire spine in all patients. Roentgen ray findings (recorded in Table 1) indicated positive evidence of the usually accepted changes characteristic of this disease. The urinary 17-ketosteroid excretion in milligrams per 24 hours is given in Table 1 and presented graphically in Figures 1 to 6. The method of determining urinary 17-ketosteroids was adapted from that by Robbie and Gibson, and described in our preliminary report (8). The x-ray therapy consisted of 6 daily treatments in each course. The cervico-dorsal and lumbo-

sacral areas were treated on alternate days using the following factors: 200 KV; 20 milliamps; 70 cm. anode skin distance; 1.0 mm. copper, half-value layer; and 15 X 8 cm. area of the average field treated. These courses of therapy were repeated in one month and again in three months.

17-KETOSTEROID EXCRETION

The average urinary 17-ketosteroid excretion in 24 hours by normal males is 14 mg.; by females, approximately 10 mg. (2, 3, 13, 14). The av-

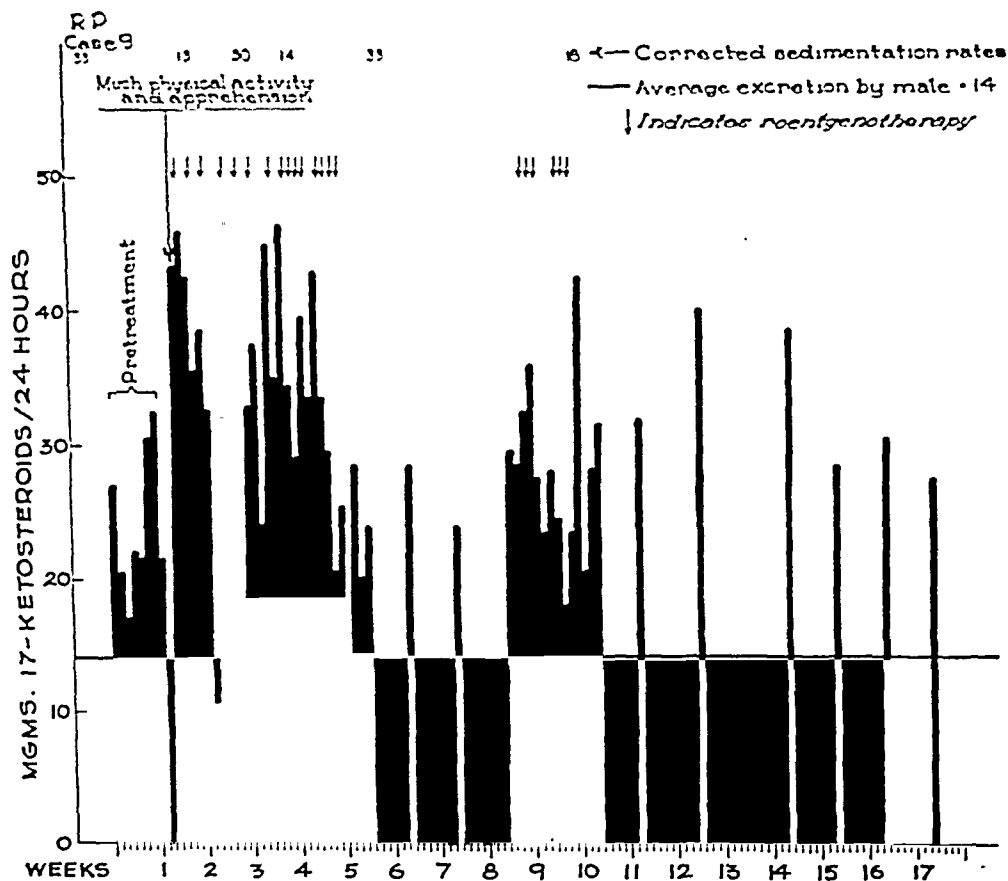


FIG. 2. R.P., case 9. Daily estimations of 17-ketosteroid excretion before, during and after roentgenotherapy.

erage excretion by the males in the spondylarthrititis group was 26.7 mg. in 24 hours (Fig. 1). High values were observed in patients before and after roentgenotherapy. Only when there was great malnutrition and a generally poor physical state (W.G., case 25) did the excretion fall to low val-

ues. When W.G. improved clinically following roentgenotherapy, his excretion increased to levels well above normal averages (Fig. 4). Patients who showed few signs of clinical activity and slow progression of the disease had levels which could be in the range of normal excretion values. Patients with evidence of rapidly progressing disease, even when the history indicated short duration, had rather high excretion values.

R.P., case 9. Figure 2, depicts the daily 17-ketosteroid excretion over a period of seventy days in this 25-year-old male who had been ill for less

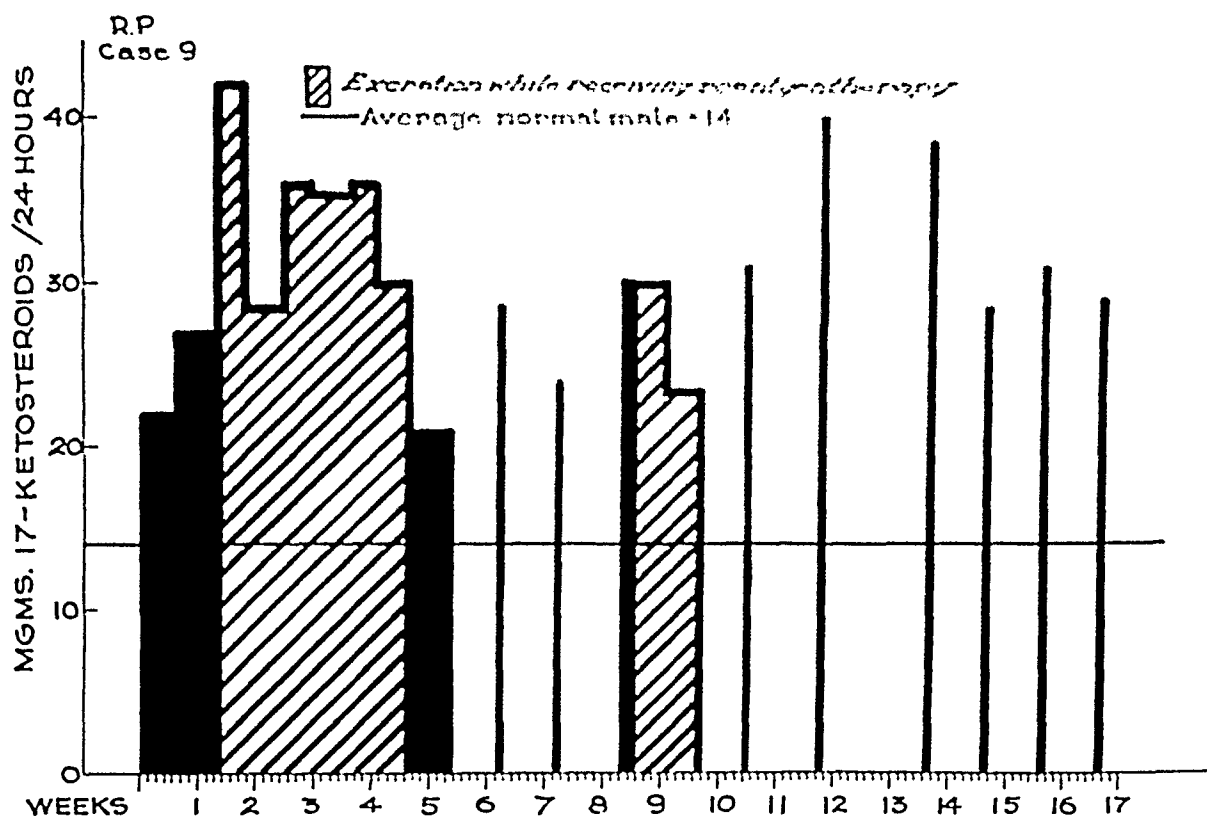


FIG. 3. R.P., case 9. Composite of Figure 2 showing 17-ketosteroid excretion in relation to days of roentgenotherapy.

than a year but was rapidly progressing to total invalidism. He had complete obliteration of the lumbar lordotic curve, marked spasm of the erector spinae muscles, great limitation of spine movement, chest expansion of one-half an inch, and the characteristic gait of such patients. Roentgenograms showed evidence of the disease only in the sacroiliac joints. Upon completion of the first course of roentgenotherapy symptoms were completely relieved, spinal movement became free, and chest expansion increased to three inches. Increased 17-ketosteroid excretion occurred the day preceding roentgenotherapy, coincident with anxiety and extra physical activity, a phenomenon which has been previously observed. A pro-

nounced fall in excretion occurred coincident with side effects of the treatment: nausea, consequent inactivity of the patient, diminished fluid and food intake. The urine volume during that 24 hours was only 750 cc. after a previous average of 1500 cc. Chou (2) and Forbes (3) have commented on the variability of 17-ketosteroid excretion in sick individuals compared with the relative constancy of excretion by normal persons. Figure 3 shows averages of excretion for several consecutive days in R.P.

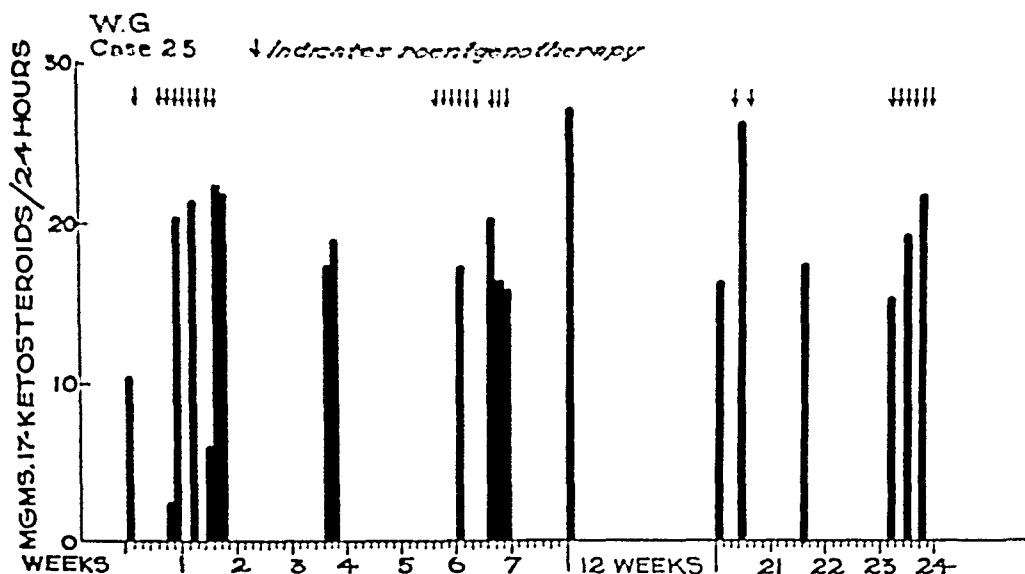


FIG. 4. W. G., case 25. 17-Ketosteroid excretion before, during and after roentgenotherapy—150 rhesmas (skin roentgens) on days shown.

These block out the daily variations and thereby give a better picture of the series of events in Case 9.

W.G. (case 25, Figure 4) was admitted to the hospital by ambulance. He was unable to walk, had severe malnutrition, muscle atrophy, spinal rigidity, and mental depression. Roentgenograms showed advanced changes characteristic of spondylarthritis throughout the spine. As Figure 4 demonstrates, the initial urine content of 17-ketosteroids was only 10.5 mg. in 24 hours' excretion. Lower levels of excretion followed roentgenotherapy. Clinical improvement and relief of pain resulted from the first course of roentgenotherapy, and 17-ketosteroid values returned to levels above the average for normal males.

L.N. (case 7, Figure 5): In this patient there appeared to be no increased 17-ketosteroid excretion following the roentgen stimulus. The variations in excretion furnished no conclusive evidence of x-ray effect. No urine specimens could be obtained between the second and third courses of roent-

genotherapy. When the patient returned for the third course, he stated he had been entirely free of symptoms following the second course of treatment. At this time it is seen there were higher excretion values than when the patient was first observed.

W.P. (case 1, Figure 6): This patient, a robust, well-nourished white male, had only minimal physical signs and mild symptoms of spondylar-

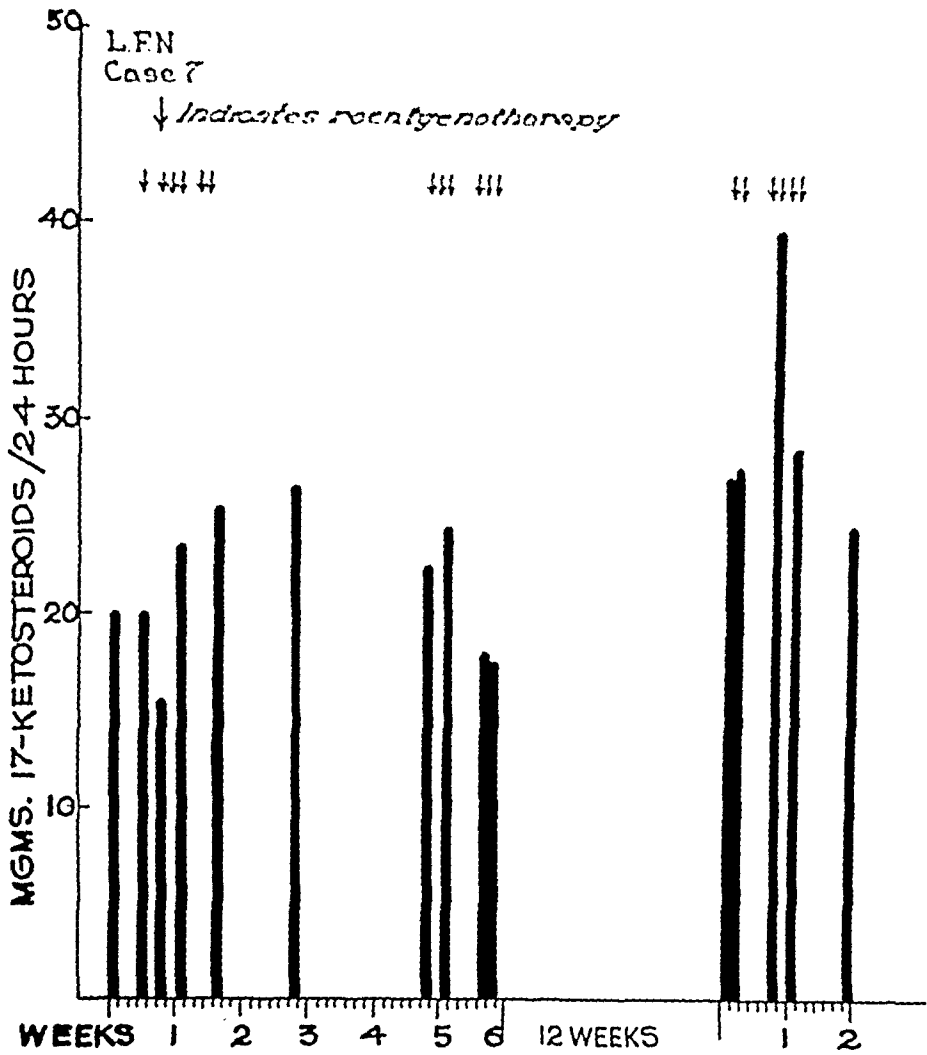


FIG. 5. L.N., case 7. 17-Ketosteroid excretion before, during and after roentgenotherapy—150 rhesmas (skin roentgens) on days shown.

thritis. The moderately high 17-ketosteroid excretion was enhanced initially coincident with roentgenotherapy and a further increase occurred during the second course of treatment.

In each of cases 12, 15, and 31 urine specimens were collected and pooled for three days. Estimations were then made on the total amount, and the average excretion for a 24-hour period was calculated from the result obtained on the pooled specimen. No graphs are presented of these studies. It

can be said, however, that the patient in case 12 showed an average excretion for three days preceding roentgenotherapy of 34 mg. in 24 hours. During the first three days of roentgenotherapy, the urinary excretion of 17-ketosteroids averaged 32.5 mg. The succeeding 3-day pools averaged

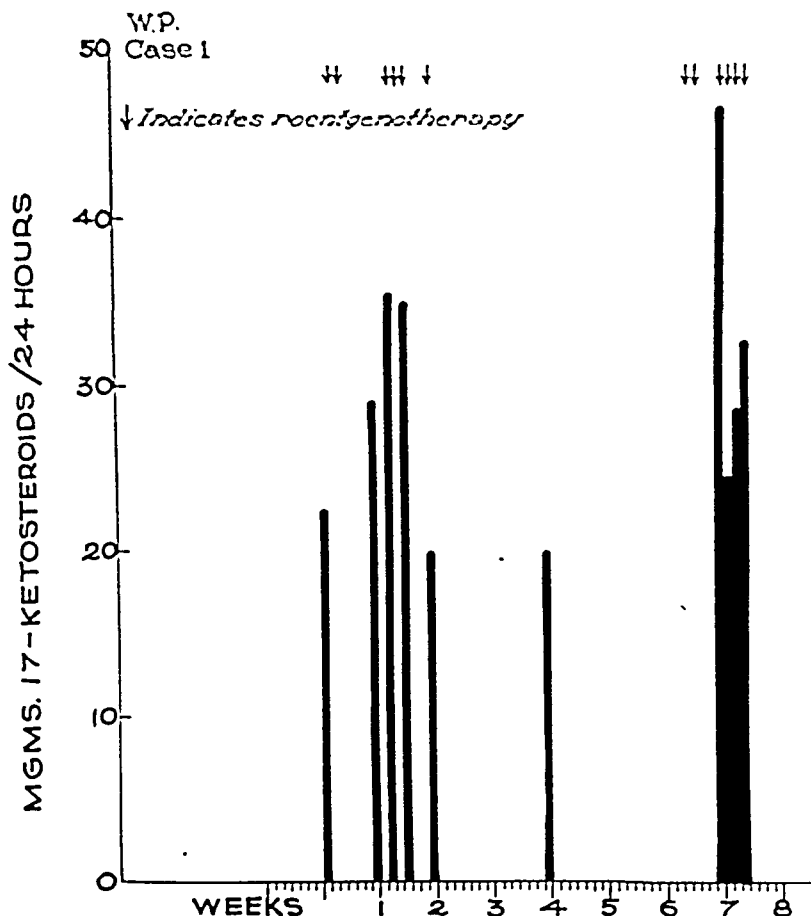


FIG. 6. 17-Ketosteroid excretion before, during and after roentgenotherapy—150 rhenas (skin roentgens) on days shown.

24 mg., 32 mg., 26 mg., 37 mg., and 29 mg. At the end of the first course of treatment and during the following three weeks no definite improvement in symptoms occurred; nor was there any lessening in severity of physical signs. Stiffness and muscle spasm continued. G.E., case 15, had an initial 17-ketosteroid excretion of 34.5 mg. in 24 hours, although the patient was acutely ill and had a hematocrit reading of 38 per cent and a corrected sedimentation rate of 35 mm. at that time. Roentgenotherapy was given every other day. The 17-ketosteroid excretion after the first treatment increased to an average of 42 mg. in 24 hours for the three-day pool. How-

ever, after three treatments, excretion fell first to the pretreatment level, and then to 13 mg.

SUMMARY

Urinary 17-ketosteroid excretion was found to be increased in spondylarthritis of the ankylosing type in both male and female patients.

High values continued for long periods of time and approached lower levels only when the patient reached a state of exhaustion. Roentgenotherapy seemed to provide a stimulus for increased excretion followed by a relative decrease. Following the roentgenotherapy the values returned to high levels although the symptoms and signs of the disease had abated.

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THE EFFECT OF VITAMIN E IN THE MENOPAUSE

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THE value of estrogens in the treatment of the symptoms of the menopausal syndrome is firmly established; however, as is well known, there are several contraindications to their use. For instance, most clinicians believe that estrogenic therapy should be avoided in the treatment of women with a familial or personal history of mammary or genital malignancy. Furthermore, in patients who are suffering from precancerous lesions estrogens seem contraindicated. In such cases and when sedation fails, physicians must resort to other therapeutic agents.

Testosterone compounds (1, 2) and a synthetic vitamin E preparation (3) have been employed. Since testosterone propionate has not been effective in our experience, we decided to determine the merits of synthetic vitamin E in combating the symptoms of the menopause.

It is the purpose of this paper to report our observations.

MATERIAL AND METHOD

Sixty-six menopausal women whose ages ranged from 35 to 68 years were studied. The patients presented themselves with the usual train of symptoms such as hot flashes and sweats, vertigo, headaches, parasthesias, fatigue, insomnia and nervousness. In 59 of the women it was deemed advisable to avoid estrogenic therapy because of the clinical findings or the history. Vitamin E¹ therapy therefore was instituted (Table 1).

In the remainder of the patients there was no contraindication to the use of estrogens, and vitamin E was administered either preceding or following estrogenic therapy for the purpose of comparing the results.

The daily dose of vitamin E ranged from 20 to 100 mg.: the average was 30 mg. in divided doses. Therapy was continued for periods ranging from ten days to seven months, with an average duration of thirty-one days. The total dose of vitamin E administered ranged from 280 to 6,300 mg.

In order to rule out a psychologic influence, placebo tablets simulating the vitamin E tablets in all respects were substituted in 17 cases.

Vaginal smears were studied in 45 patients with the purpose of determining any possible hormonal effect on the vaginal epithelium.

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¹ Synthetic vitamin E, in the form of tablets of Ephynal Acetate, 10 mg. each, were supplied through the courtesy of Dr. Elmer Sevringhaus and Dr. Leo Pirk of Hoffmann-La Roche, Inc., Nutley, New Jersey.

TABLE 1. CLINICAL FINDINGS OR HISTORY MILITATING AGAINST THE USE OF ESTROGENS IN 59 MENOPAUSAL PATIENTS

	No. of patients
Uterine fibroids	15
Postradiation castration	4
Cervical carcinoma	2
Carcinoma of breast	2
Chronic cystic mastitis	1
Functional uterine bleeding	2
Uterine bleeding induced by estrogens	5
Prolonged estrogenic therapy	7
Intolerance to estrogens	3
Cervical polyp and endometrial hyperplasia	1
Advanced age (60-68 years)	7
Leukoplakia vulvae	2
Neuromuscular disturbances	3
Premenopausal state	5

are classified as excellent, good, fair or unsatisfactory, according to definite standards. The response was considered excellent when there was complete relief of symptoms and an added sense of well-being; results were classified as good when there was almost complete relief of symptoms and a moderate sense of well-being; and as fair when there was only partial relief of symptoms. When no relief was obtained, the results were termed unsatisfactory.

As seen in Table 2, 31 patients or 47 per cent obtained good to excellent results; 16 or 24.2 per cent obtained fair relief, and 19 or 28.8 per cent were not benefited. Among the latter there were 5 patients who complained of aggravation of symptoms. In two others side effects occurred, which con-

RESULTS

The results obtained with vitamin E therapy are listed in Table 2. They

TABLE 2. RESULTS OBTAINED IN 66 MENOPAUSAL PATIENTS TREATED WITH VITAMIN E

	No. of patients	Per cent of patients
Excellent	5	7.6
Good	26	39.4
Fair	16	24.2
Unsatisfactory	19	28.8

sisted mainly of ocular symptoms (burning of the eyes, spots before the eyes, blurred vision and heaviness of the eyelids).

Two illustrative cases are presented; one demonstrating the beneficial results of vitamin E therapy with placebo control and the other, the failure of vitamin E therapy.

Case E.W. (No. 31 in Table 3), a 48-year-old married woman, was seen on July 22, 1947 with the chief complaints of hot flashes, sweats and headaches of four years' duration. These followed the use of radium for cervical carcinoma and were increasing in intensity to the point where they were occurring 8 to 10 times daily. Physical examination findings were normal and revealed no evidence of the previous cervical carcinoma. Vaginal smears were also free of carcinoma cells. Due to the previous history of carcinoma, estrogenic therapy was contraindicated and therefore the patient was placed on synthetic vitamin E therapy, 10 mg. t.i.d., p.c. Within a week the patient reported improvement in the severity and decrease in the frequency of the symptoms. When the medication was increased to 20 mg. t.i.d., there was a complete disappearance of her hot flashes and sweats. After five weeks of vitamin E therapy, it was decided to substitute placebo medication in order to evaluate the role which psychogenic influence might have played. There was a prompt recurrence of her original complaints within a week. Re-institution of vitamin E therapy once again caused a subsidence of the complaints.

Case Y.F., a 43-year-old unmarried woman, was seen on August 14, 1947, with the chief complaint of hot flashes and sweats of two years' duration, following the use of radiation therapy for a uterine fibroid with meno-metrorrhagia. Although estrogens had controlled these menopausal symptoms, this medication had to be discontinued periodically because of recurrence of moderate bleeding. Vitamin E (10 mg. t.i.d.) was administered for two weeks. Although the bleeding stopped, hot flashes and sweats recurred. An increase of the dosage of vitamin E to 20 mg. t.i.d. for another three-week period also had no therapeutic effect. Vitamin E was discontinued and sedatives were substituted, with partial relief of symptoms.

Those patients who benefited from vitamin E therapy and who later discontinued the medication experienced a recurrence of all their symptoms after a period of from three days to one month. Reinstitution of vitamin E therapy resulted again in relief of symptoms. Placebo medication was substituted for the vitamin E preparation in 17 patients who had obtained good results from vitamin E therapy. All had a recurrence of symptoms and relief ensued when vitamin E was reinstituted (Table 3). It is important to note that 3 patients who were not included in this series continued to claim good results with inert material, after vitamin E was withdrawn.

TABLE 3. EFFECT OF PLACEBO SUBSTITUTION IN 17 PATIENTS TREATED WITH VITAMIN E

Case No.	Age Yrs.	Symptoms	Vitamin E therapy			Placebo therapy	
			Duration	Total amount	Results	Duration	Results
#16 A.M.	68	Hot flashes, sweats, chills	2 months	mg. 1800	Excellent	2 weeks	Recurrence of symptoms
#17 E.M.	48	Hot flashes, menorrhagia	2 weeks	420	Good	"	"
#18 D.V.	43	Headaches, hot flashes, vertigo, menorrhagia	3 weeks	630	Good	"	Recurrence of symptoms on 3 different occasions
#20 C.M.	47	Hot flashes, sweats, headaches	2 weeks	420	Good	"	Recurrence of symptoms
#21 G.S.	49	Hot flashes, vertigo, fatigue, irritability	4 weeks	900	Excellent	"	"
#23 K.V.	48	Hot flashes, sweats	2 weeks	420	Good	"	"
#30 K.D.	51	Hot flashes	7 weeks	2940	Good	"	"
#31 E.W.	48	Hot flashes, sweats (postradiation)	3 weeks	1684	Excellent	1 week	"
#33 K.R.	50	Hot flashes, headaches, vertigo	4 weeks	1200	Fair	2 weeks	"
#37 B.W.	50	Hot flashes, sweats, numbness, headaches	3 weeks	630	Fair	"	"
#38 P.Z.	50	Hot flashes	3 weeks	630	Good	"	"
#39 A.M.	48	Hot flashes, headaches, crying spells (postradiation)	2 weeks	420	Good	"	"
#43 H.J.	59	Hot flashes	4 weeks	840	Good	"	"
#55 H.P.	50	Hot flashes, headaches, fatigue, parasthesia, emotional disturbances	3 weeks	630	Good	"	"
#58 K.B.	46	Headaches, insomnia, dyspnea, menorrhagia	6 weeks	2520	Good	"	"
#59 G.G.	46	Hot flashes, sweats, headaches, insomnia, parasthesia	3 weeks	630	Good	"	"
#64 E.K.	57	Hot flashes, sweats, headaches	6 weeks	1260	Good	"	"

Physical examination revealed no changes in the breasts or the uterus in any of the patients. Similarly, no effect on the vaginal epithelium was detected by studying the vaginal smear picture according to the method developed by Papanicolaou. Therefore, with this type of therapy, the vaginal smear cannot be utilized as an index of the therapeutic effect.

DISCUSSION

As mentioned previously, a familial or personal history of malignant disease constitutes a contraindication for the use of estrogens. Also, it seems wise to avoid them in patients with precancerous conditions with uterine fibroids, cervical polyps, fibroadenomata of the breast or chronic cystic mastitis. Furthermore, there are women in whom the menopausal syndrome is characterized by meno-metrorrhagia; and women in whom the administration of follicular hormone produces vaginal bleeding. In these groups also, estrogenic therapy should not be employed. In addition, it has been reported (4) that patients of advanced age (past 60) may develop pathologic tissue changes under the influence of prolonged estrogenic therapy. Finally, in that group of patients with menopausal vasomotor disturbances who still have normal cycles, it is inadvisable to use estrogens because of the danger of producing prolonged irregular menses.

Christy (3) was the first to use vitamin E in the menopause. He employed this therapy in an attempt to relieve menopausal discomfort in 25 patients with a definite diagnosis or a strong suspicion of neoplastic disease. According to him, "the entire group of cases responded to the treatment and showed either complete relief or very marked improvement with less frequency and less severity of the hot flashes and drenching perspiration, and a definite change for the better in their mood and outlook." Christy also states that in some of the cases relief of vasomotor instability was more easily obtained with the use of vitamin E than with the estrogens. While we are in agreement with this investigator that vitamin E constitutes a valuable means of controlling menopausal symptoms, it certainly is not effective in all cases as can be seen in Table 2. We also feel that estrogens give more rapid and more complete relief than vitamin E in most cases. This observation was made in the 7 cases in which vitamin E was administered either preceding or following estrogens for the purpose of comparison. However, vitamin E therapy is the medication of choice in cases in which the use of estrogens is contraindicated.

SUMMARY AND CONCLUSIONS

1. Vitamin E therapy was instituted in 66 selected and controlled patients who complained of the characteristic vasomotor symptoms of the

menopause. Good to excellent results were obtained in 31 women, and fair results in 16. In 19 patients the treatment was ineffectual.

2. Discontinuance of the vitamin E preparation was attended by prompt recurrence of symptoms with relief resulting again on reinstitution of the medication.

3. Substitution of placebo medication for the vitamin E preparation in 17 patients caused a recurrence of symptoms.

4. No changes in the breasts, uterus or vaginal epithelium were noted during vitamin E therapy. Side effects were negligible and there were no contraindications to its use.

5. Vitamin E is a valuable aid in the treatment of menopausal patients in whom estrogens are contraindicated.

Acknowledgment

I gratefully acknowledge the cooperation of my associates on the Endocrine Service, Drs. Zelda Marks, George M. Cohn, Gertrude Oberlander, and Sylvia F. Becker.

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A RAPID COLORIMETRIC METHOD FOR THE DETERMINATION OF SODIUM IN BIOLOGICAL FLUIDS

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GILBERT C. H. STONE, Ph.D.

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THE clinical importance of sodium metabolism becomes increasingly evident with every year. However, widespread investigation in this field has been severely restricted because of the absence of a generally useful method for sodium determination, i.e., one which requires no specialized equipment, unusual technical skill, or great expenditure of time and which may therefore be fitted into the routine of the general clinical laboratory.

The speed and sensitivity of the flame photometer, for instance, are largely offset by its capriciousness: the problem of flame stability has not yet been overcome, with the result that the reproducible accuracy in expert hands is only 5 to 10 per cent and that "frequently an hour or more is required to establish conditions of equilibrium for the desired range of concentration" (1). Also, the cost of such instruments at the present time may be prohibitive. A sensitive nephelometric technique has been described (2) but this seldom-used method also requires an instrument which is not commonly available. Purely chemical methods are based on the formation of relatively insoluble acetates of sodium with uranium and zinc or uranium and magnesium, having the formulae $\text{NaZn}(\text{UO}_2)_3(\text{CH}_3\text{COO})_9 \cdot 9(?)\text{H}_2\text{O}$ and $\text{NaMg}(\text{UO}_2)_3(\text{CH}_3\text{COO})_9 \cdot 9(?)\text{H}_2\text{O}$. Modifications (3, 4, 5) of the older gravimetric technique for measuring the triple salt have speeded up the procedure, though sometimes with a great sacrifice of sensitivity (4). The otherwise excellent method of Consolazio and Dill (5) requires ashing, which is a long and tedious process.

All these chemical methods are limited by the same basic obstacle, namely the low color intensity of the sodium triple salt. Recognizing this, color intensification with potassium ferrocyanide (6) has been attempted, but the ferrocyanide reaction has been criticized repeatedly as being highly susceptible to changes in temperature and to excesses of the reagents. The use of ammonium thiocyanate (7) serves only to stabilize the color of the triple salt, but has no intensifying action.

In 1929 Rosenheim and Daehr (8) found that an intense yellow-to-red color was produced by the action of hydrogen peroxide on alkaline solutions

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of the uranyl ion. This reaction was studied by Arnold and Pray (9) who found that it followed Beer's law and was therefore suitable for quantitative spectrophotometry. In the course of our investigation we were able to corroborate this aspect of their work. Even though the exact nature of the hydrated uranyl oxide ion in alkaline solution is unknown, it has been possible to use it in developing a rapid and extremely sensitive colorimetric method suitable for biological fluids.

METHOD

Reagents

1. Uranyl zinc acetate reagent. Add 77 Gm. of uranyl acetate to 750 ml. distilled water and 14 ml. glacial acetic acid which have been heated nearly to boiling. To this mixture is added 231 Gm. of zinc acetate, divided into five or six portions, with frequent stirring. Finally, 7 ml. of glacial acetic acid is added and the solution is allowed to cool. After cooling, the reagent is diluted to 1 liter with distilled water and 200 ml. of 95 per cent ethyl alcohol is added. The solution is refrigerated overnight, filtered, and is then ready for use.

2. Sodium triple salt. To 10 ml. of the above solution is added an excess of a concentrated solution of sodium chloride. The precipitate is collected by filtration, washed five times with glacial acetic acid and five times with ether.

3. Wash reagent. A solution of 425 ml. of 95 per cent ethyl alcohol and 75 ml. glacial acetic acid is saturated at room temperature with the sodium triple salt (see above). This solution is kept in a brown bottle.

4. A saturated solution of mercuric chloride in 95 per cent ethyl alcohol.

5. A saturated aqueous solution of ammonium carbonate.

6. A 30 per cent solution of hydrogen peroxide ("Superoxol").

7. Powdered calcium hydroxide.

Filtrates

1. Urine. To approximately 10 ml. urine add 3 drops of mercuric bichloride solution, shake and let stand for a few minutes. Then add a spatula-tipful of calcium hydroxide powder, shake well, then filter.

2. Serum. To 3.0 ml. of 10 per cent trichloroacetic acid add 1.0 ml. serum, dropwise. Shake, and centrifuge or filter.

Procedure

Pipette 0.25 ml. of the urine filtrate or 1 ml. of the serum filtrate into a 15 ml. centrifuge tube and add 5 ml. of the uranyl zinc reagent. Mix by twirling the tube and allow to stand for 20 minutes. The tubes should be agitated briefly at least twice during this interval. Centrifuge at high speed for 7 minutes, then decant the supernatant fluid and drain for 1 minute. Expel 5 ml. of the wash reagent onto the precipitate, washing down the sides of the centrifuge tube during the process. Centrifuge for 7 minutes, decant and drain. Dissolve the remaining precipitate with a few drops of distilled water, add 6 ml. of the saturated ammonium carbonate solution and 1 ml. of the 30 per cent hydrogen peroxide in that order, and dilute to 15 ml., or to 25, 50 or 100 ml. by transfer to a suitable volumetric flask with washings. The appropriate final dilution (i.e., 15, 25, 50 or 100 ml.) depends on the sensitivity of the colorimeter to be used. The correct dilution for the individual instrument is the one which gives to the desired range of values the greatest

spread on the meter or dial of the instrument.) Mix by inversion and read in the colorimeter using a filter with maximum transmittance at 415-430 $m\mu$.

EXPERIMENTAL DATA

Absorption spectrum

The absorption spectra of two different concentrations as determined with a Beckman spectrophotometer model DU are shown in Figure 1. These curves are quite different in shape from those of Arnold and Pray, who found transmittance minima in the region of 400 to 460 $m\mu$. The curves obtained by us show no minima even though measurements were carried almost into the ultraviolet. This difference in the absorption spectra is in all

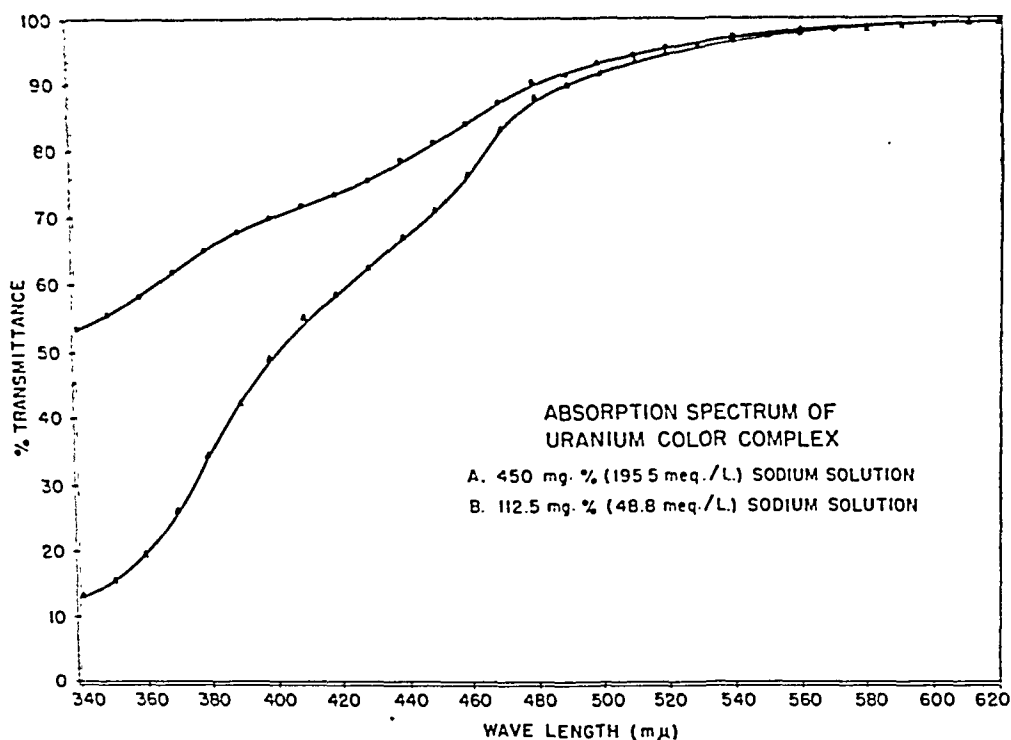


FIG. 1

probability due to the different dilutions employed by Arnold and Pray and ourselves. Their two curves represent 0.3 and 1.3 mg. of sodium respectively whereas ours, recalculated in terms of their technique, represent 0.09 and 0.0225 mg. On the basis of Figure 1 it is evident that the shorter the wavelength (in the visible region), the greater the sensitivity. For practical application in colorimeters, filters with a maximum transmittance in the region of 415 to 430 $m\mu$ are readily available and have been found entirely satisfactory.

A calibration curve is shown in Figure 2. Calibrations have always been carried out by precipitating and developing standard solutions of sodium (chloride) in 6 per cent trichloroacetic acid, according to the method described. In the range of serum sodium concentrations, the sensitivity is 11 mg. per cent (4.8 mEq./L.) per scale division, using a Leitz-Rouy col-

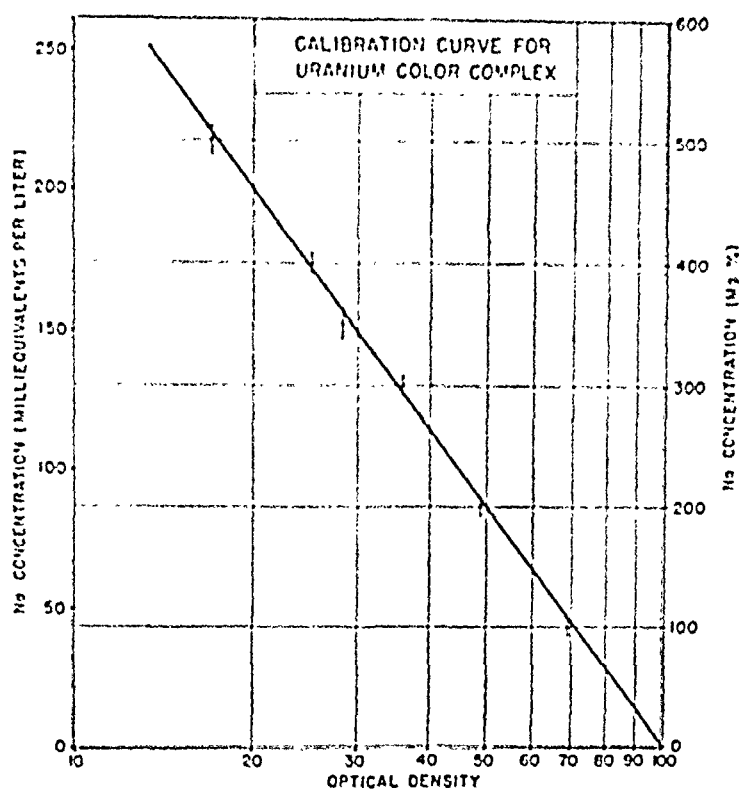


FIG. 2

orimeter. Estimates of a tenth of a scale division are made with ease, making the theoretical sensitivity 1.1 mg. per cent (0.5 mEq./L.).

Reproducible accuracy

This was studied by numerous triplicate determinations, a typical example of which is reproduced in Table 1. In some cases, known dilutions of the sodium triple salt were developed for color and in others, various known amounts of sodium were added to serum and carried through the entire procedure (Table 1). Data obtained by both these methods indicate a reproducible accuracy of 3 per cent.

Procedure

The substances which interfere with quantitative triple salt formation are protein, phosphate, arsenate, lithium, and large amounts of potassium

(10). In biological fluids, only the protein and phosphate are of importance.

Removal of protein from serum and spinal fluid is accomplished by the use of trichloroacetic acid. Since serum inorganic phosphate introduces a constant absolute error of only 1 mEq./L. (11) with proportionately small changes due to variation in the serum phosphate level, this factor is usually ignored.

In urine, mercuric chloride is used for protein precipitation; trichloroacetic acid would of course be incompatible with the calcium hydroxide. Some workers (2) have found that refrigeration is adequate for the removal of

TABLE 1

Sample	Meter reading	Observed (mEq./L.)	Calculated (mEq./L.)	Error (per cent)
1.0 ml. serum	32.5			
#1-S3	34.9			
	33.0			
	(av. 33.5)	137		(av. 2.4)
1.0 ml. serum	29.9	151		1.9
#1-S3 plus 40 mg. (17 mEq.) sodium	30.0	150	154	2.6
	32.0	147		4.5
0.5 m. serum	33.0	138		3.0
#1-S3 plus 150 mg. (65 mEq.) sodium	33.1	138	134	3.0
	33.1	138		3.0

urinary phosphate. However, this procedure is time-consuming. We have found that calcium hydroxide serves the purpose far better, for it requires no extra time and does not complicate matters by causing changes of urine volume due to temperature.

The efficiency of washing was studied by means of 10 pairs of precipitates, half of which were washed once with 5 ml. and half twice with 2.5 ml. of the wash reagent. The greater efficiency of the double washing could not be observed by the usual technique in the colorimeter and required special means (a 6 cm. column of fluid) for its demonstration. Another group of 10 precipitates was washed twice with 5 ml. portions of the wash reagent. No perceptible increase in color density of the second washing over that of unused wash reagent could be demonstrated.

With proper centrifuging, no difficulty was encountered in packing the precipitate firmly, and with these small amounts of precipitate no troublesome flotation of crystals (as found by Bradbury) occurred.

The developed color was tested for stability and no changes were observed during a 2-hour observation period. However, the formation of oxygen bubbles frequently takes place fairly soon after the addition of the hydrogen peroxide. Vigilance must be exercised to dislodge all bubbles before readings are carried out, and it was found advantageous to carry out the readings as promptly as possible in order to avoid this nuisance.

SUMMARY

A rapid colorimetric method for the determination of sodium in biological fluids is presented. The method is based on the precipitation of sodium as the triple salt of uranium and zinc acetate, and intensification of the color of the uranyl ion with hydrogen peroxide in alkaline solution. The reproducible accuracy of the method is 3 per cent.

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The Association for the Study of Internal Secretions Announces A Postgraduate Assembly In Endocrinology

OKLAHOMA CITY, OKLAHOMA SKIRVIN HOTEL FEBRUARY 21-26, 1949

The faculty will consist of prominent researchers and clinical endocrinologists in the various branches of the medical sciences, gathered from the United States and Canada and will include the following:

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| Dr. Willard M. Allen, Professor and Head, Department of Obstetrics and Gynecology, Washington University School of Medicine. | Dr. Harold L. Mason, Professor, Physiological Chemistry, Mayo Foundation, University of Minnesota. |
| Dr. Edwin B. Astwood, Research Professor of Medicine, Tufts College. | Dr. Warren O. Nelson, Professor of Anatomy, University of Iowa College of Medicine. |
| Dr. J. S. L. Browne, Professor of Medicine, McGill University. | Dr. Edward Ryncarson, Associate Professor of Medicine, Mayo Foundation, University of Minnesota. |
| Dr. Edward A. Doisy, Professor and Head, Department of Biochemistry, St. Louis University School of Medicine. | Dr. Hans Selye, Professor of Experimental Medicine, University of Montreal. |
| Dr. Roberto Escamilla, Associate Clinical Professor of Medicine, University of California Medical School. | Dr. E. Kost Shelton, Associate Professor of Medicine, University of Southern California. |
| Dr. E. C. Hamblen, Associate Professor of Obstetrics and Gynecology, Duke University School of Medicine. | Dr. Paul M. Starr, Clinical Professor of Medicine, University of Southern California. |
| Dr. Laurence W. Kinsell, Associate Clinical Professor of Medicine, University of California Medical School. | Dr. Willard O. Thompson, Clinical Professor of Medicine, University of Illinois College of Medicine. |
| Dr. C. N. H. Long, Sterling Professor of Physiological Chemistry and Dean, Yale University School of Medicine. | Dr. George Thorn, Hershey Professor of the Theory and Practice of Physic, Harvard Medical School. |
| Dr. Cyril M. MacBryde, Associate Professor of Clinical Medicine, Washington University School of Medicine. | Dr. Henry H. Turner, Associate Professor of Medicine, University of Oklahoma School of Medicine. |
| Dr. E. Perry McCullagh, Chief, Dept. of Endocrinology and Metabolism, Cleveland Clinic. | Dr. Lawson Wilkins, Associate Professor of Pediatrics, Johns Hopkins Hospital. |

This course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

A fee of \$100 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED. Should there be an insufficient number of applicants to fill the course, the registration fee will be immediately refunded in its full amount.

Please forward application on your letterhead, together with check, payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Chairman of the Postgraduate Committee, 1200 North Walker Street, Oklahoma City, Oklahoma, before February 1, 1949.

Applicants should make reservations directly with hotels of their choice. Some of the better downtown hotels in Oklahoma City, listed according to their proximity to the Skirvin are: Skirvin Tower, Huckins, Wells-Roberts, Biltmore and Black.

ANNOUNCEMENT OF NATIONAL RESEARCH COUNCIL GRANTS FOR RESEARCH IN ENDOCRINOLOGY

The Committee on Research in Endocrinology, National Research Council, wishes to announce that requests for grants-in-aid during the fiscal period from July 1, 1949, to June 30, 1950, will be received until February 28, 1949. Application blanks may be obtained by addressing the Secretary, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C. In addition to a statement of the problem and research plan or program, the Committee desires information regarding the proposed method of attack, the institutional support of the investigation and the uses to be made of the sum requested. No part of any grant may be used by the recipient institution for administrative expenses.

The Committee makes grants-in-aid of research in the general field of experimental and clinical endocrinology. However, applications for support of research in the problems of sex in the narrower sense cannot be given favorable consideration, and investigators seeking support in this field should direct their proposals to the Committee for Research in Problems of Sex of the National Research Council. The Committee on Research in Endocrinology, however, will continue to give consideration to the support of studies of the effect of sex hormones on non-sexual functions, *e.g.*, on general metabolism and on the metabolism of steroid hormones.



The 1949 Meeting of the Association for the Study of Internal Secretions

The Thirty-First Annual Meeting of the Association for the Study of Internal Secretions will be held in the Chalfonte-Haddon Hall, Friday and Saturday, June 3 and 4, 1949, in Atlantic City, New Jersey.

We are informed by the hotel management that reservations will be difficult to secure on short notice; therefore, members are urged to make reservations at once with Chalfonte-Haddon Hall, giving time of arrival and length of stay in Atlantic City.

The scientific sessions will be held in the Viking Room, as formerly, and registration will be on the same floor. The annual dinner will be held in the Rutland Room, Friday, June 3, at 7 p.m., preceded by cocktails in the same room.

Those wishing to present papers, which will be limited to ten minutes, should send the title and four copies of an abstract of not more than 200 words, to Dr. J. S. L. Browne, Royal Victoria Hospital, Montreal 2, Canada, not later than March 1, 1949. It is imperative that the abstracts be informative and complete, with results and conclusions, in order that they may be of value for reference and suitable for printing in the program.

Nominations for the Squibb and Ciba Awards and the Ayerst, McKenna and Harrison Fellowship should be filed on special forms with the Secretary of the Association, not later than March 15, 1949, according to specifications given in the section on Awards.

The Awards of the Association for 1949

Nominations for Awards

Three Awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each Award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma. All nominations, accompanied by a statement of the importance of the nominee's contributions to endocrinology and a bibliography of his most important papers with reprints if possible, should be sent to Dr. Turner's office not later than March 15, 1949.

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of \$1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. Edward A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori and in 1948 to Dr. Fuller Albright. No award was made in 1943. No age or special limitation is stipulated by the donor of the Award.

THE CIBA AWARD

The Ciba Award, established in 1942, is given in recognition of the meritorious accomplishment of an investigator, not over 35 years of age, in the field of clinical or preclinical endocrinology. In 1944 the Award was given to Dr. E. B. Astwood; in 1945 to Dr. Jane Anne Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li and in 1948 to Dr. Carl G. Heller. The Award is for \$1,200.00. If within two years of the date of the Award, the recipient chooses to use it to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to \$1,800.00.

THE AYERST, McKENNA & HARRISON FELLOWSHIP

The first Ayerst, McKenna & Harrison Fellowship was given to Dr. Samuel Dvoskin in 1947 and in 1948 it was given to Dr. Ernest M. Brown, Jr. This Fellowship is designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. The Fellowship may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend for the Fellowship will vary in accordance with the qualifications of the appointee, but will not exceed \$2500.00. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals, submit a program of proposed study, indicate one or more institutions where the proposed program shall be carried out and submit statements of approval from the investigators with whom he proposes to conduct his research.



American Goiter Association

THE 1949 MEETING

The next annual meeting of the American Goiter Association will be held at the Loraine Hotel in Madison, Wisconsin, May 26, 27 and 28, 1949.

All members of the Association are urged to make their hotel reservations as early as possible. Those who would prefer a resort hotel will find the new Edgewater very beautiful. It is half a mile from the Loraine on the shores of Lake Mendota.

The officers of the Society are as follows:

President	—Arnold S. Jackson, Madison, Wisconsin
President Elect	—Samuel F. Haines, Rochester, Minnesota
Vice President	—Willard O. Thompson, Chicago, Illinois
Corresponding Secretary	—T. C. Davison, Atlanta, Georgia
Recording Secretary	—Geo. C. Shivers, Colorado Springs, Colorado
Treasurer	—V. E. Chesky, Halstead, Kansas

Executive Councilors

J. H. Means, Boston, Massachusetts
Elmer Bartels, Boston, Massachusetts
Allen Graham, Pittsburgh, Pennsylvania
H. P. Sloan, Bloomington, Illinois
D. U. McGregor, Hamilton, Ontario, Canada

Dr. George Crile, Jr. of Cleveland is Chairman of the Program Committee.

THE VAN METER PRIZE

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Madison, Wisconsin, May 26, 27 and 28, 1949, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed three thousand words in length, and must be presented in English. A typewritten double spaced copy should be sent to the Corresponding Secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than March 15, 1949. The Committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association. This will not prevent its further publication, however, in any journal selected by the author.

Abstracts of

CURRENT ENDOCRINE LITERATURE

Editor: ROY HERTZ. *Collaborators:* A. R. ABRAHAMSON, F. S. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, R. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

PITUITARY

KANTER, A. E., and KLAUANS, A. H.: Shock from posterior pituitary extract, *Am. J. Obst. & Gynec.* 56: 366-369, 1948.

The authors report 5 instances of shock in association with the use of posterior pituitary extract in thousands of obstetric and gynecologic patients. This shock occurs as a result of the direct pressor action upon the coronary arteries. It is readily combatted by the use of adrenalin chloride or ephedrine sulfate and infusions of fluids, whole blood or blood plasma. Because of the potentialities of shock, the authors favor the use of ergot derivatives in obstetrics. However, for use in gynecologic surgery, they believe there is no adequate substitute for posterior pituitary extract.—C.D.D.

THYROID

BOEKELMAN, A. J.: The thyroid as a regulator of potassium metabolism, *Presse méd.* 56: 23-25, 1948.

The basal metabolism is directly proportional to the potassium content of the erythrocytes. The potassium content is high in hyperthyroidism and drops with iodine treatment as the metabolic rate decreases. Iodine has no influence on the corpuscular potassium in normal persons. The potassium in the plasma is unaffected.—A.E.M.

HOLLANDER, G. H., and MANDELBAUM, H.: The treatment of angina pectoris with propylthiouracil, *Ann. Int. Med.* 28: 1150-1156, 1948.

Ten hypertensive patients with a definite anginal syndrome were studied, (1 male, 9 females). The ages ranged from 45 to 62 years. All patients had been tried on other types of medication without success. Six showed definite symptomatic improvement on 50 to 200 milligrams of propylthiouracil daily. Two of the others became progressively worse. No correlation was noted between improvement and level of the basal metabolic rate. If improvement occurred, it was manifested within 8 weeks of onset of treatment. It was not necessary to produce myxedema to get relief of pain. The authors believe that a further clinical trial of 6-propylthiouracil in angina pectoris is indicated.—C.D.D.

HORLICK, L., and HAVEL, L.: The effect of feeding propylthiouracil and cholesterol on the blood cholesterol and arterial intima in the rat, *J. Lab. & Clin. Med.* 33: 1029-1036, 1948.

In view of previous findings that the thyroid gland is involved in the entire process of

experimental atherosclerosis, propylthiouracil was administered to rats, in an attempt to break down the resistance of this species to the induction of atherosclerosis by cholesterol feeding. In contrast to findings in dogs, propylthiouracil failed to break down the rat's resistance, indicating that it may be dependent on other factors in addition to the thyroid gland and its secretions. The combination of cholesterol with propylthiouracil produced a synergistic rise in the blood cholesterol levels, an action not clearly understood.—*T.H. McG.*

MCCULLAGH, E. PERRY; HIBBS, R. E., and SCHNEIDER, R. W.: Propylthiouracil in the treatment of hypothyroidism, *Am. J. M. Sci.* 214: 545-552, 1947.

Propylthiouracil is a safe drug. In 218 patients treated for an average of 8 months and in some as long as 14 months, no cases of agranulocytosis were seen, in contrast with its occurrence in thiouracil treatment. For this reason the routine practice of taking weekly white blood counts was discontinued. Three minor reactions to propylthiouracil occurred, and 4 other reactions required the cessation of treatment. It was found effective in controlling hyperthyroidism of all types except possible instances of acute crisis in which its reaction is too slow. The hyperthyroidism of acromegaly may also be an exception. An effective dose in more than 95% of the patients was 300 mg. per day. A smaller dose was effective in many, and a larger dose was required in less than 4%. (An effective dose was defined as one which produced a progressive fall in basal metabolic rate of 2% or more per week to within normal range.) Iodine in doses as high as 30 mg. per day may be used concurrently with propylthiouracil. It will eliminate the thrill and bruit in diffuse glands and does not hinder the action of propylthiouracil. The probable frequency of permanent remissions of hyperthyroidism following the use of propylthiouracil can not yet be properly estimated. At present, long-continued use of the drug appears warranted in some patients and may later appear feasible and perhaps desirable in many.

The authors close with a tentative plan for the use of propylthiouracil to the best advantage of the patient with hyperthyroidism, with special regard to age, type of hyperthyroidism and kinds of complications.—*E.C.R., Jr.*

PETERS, J. P., and MAN, E. B.: The relation of albumin to precipitable iodine of serum, *J. Clin. Investigation* 27: 397-405, 1948.

Hypoalbuminemia associated with serum iodine deficiency in the absence of definite evidence of hypothyroidism was demonstrated in a series of patients with miscellaneous conditions (cirrhosis of the liver, craniopharyngioma, anorexia nervosa, hypopituitarism, and others) in which hypoalbuminemia appeared to be the only common factor. Administration of active thyroid substance in doses that are effective in the treatment of myxedema failed to raise the precipitable iodine in the serum of these patients; injections of enough salt-poor human albumin to raise considerably their serum albumin did not consistently increase, and more often decreased, the precipitable iodine. Data on 22 cases are tabulated.—*T.H. McG.*

WILLIAMS, R. H., and KAY, G. A.: Further studies on the correlation of chemical structure and antithyroid effect, *Am. J. M. Sci.* 213: 198-205 (Feb.) 1947.

The authors briefly review the work done since 1943, prompted by the early clinical experiences with thiouracil which indicated that serious toxic reactions sometimes oc-

curred. It was hoped that compounds could be found which have stronger antithyroid action, thereby exposing the body tissues to a smaller concentration of the drug, or substances which by their difference in chemical structure were less toxic than thiouracil even though their concentration in the body might be greater. Most of the compounds which the present authors studied were derivatives of thiourea or aniline, in addition to which a few organic sulfur compounds were tested. It was especially desirable to test the antithyroid effect of iodinated thioureas and iodinated aminobenzenes. Experimental animals were rats and chicks.

The most potent antithyroid compounds found were thiouracils with short-chain hydrocarbons in the 6-position, especially the *n*-propyl derivative. The toxicity of all of the thiouracils was relatively low and caused no significant inhibition of growth.

Of the thioureas none possessed significant activity, with the exception of 2-amino-thiazol, though to a much lesser degree than thiouracil.

The activity of several anilides structurally similar to *p*-amino-benzoic acid was relatively slight, though that of diaminobenzophenone and of *p*-aminocinnamic acid were fairly great.

The iodinated compounds showed little or no antithyroid effect, besides being relatively toxic.

Combined treatment with para-aminobenzoic acid and thiouracil proved that the amount of goitrogenesis equals, or perhaps exceeds, an additive effect of the two compounds. Many generalizations, made on correlating antithyroid activity with chemical structure, are listed and tabulated.

Finally the authors draw the following conclusions: minor structural changes in the two main series of goitrogenic compounds, thiocarbonamides and aminobenzenes, may modify the activity of these compounds, sometimes causing a marked potentiation of effect, and at other times greatly decreasing or abolishing the effect. The C=S linkage is essential, and usually without substituents attached to the sulfur. In most instances this linkage is adjacent to two amino groups, although activity can exist when one of the amino groups is replaced by an oxygen or sulfur grouping. Closure of thiourea chains generally causes a potentiation of effect. Additions of short-chain hydrocarbons tend to augment the effect of thiouracil, when attached to the 6-position. Thus far, iodination of the thiourea amino-benzene derivatives has not augmented the goitrogenic effect. The simultaneous ingestion of thiouracil and *p*-aminobenzoic acid appeared to result in an additive effect.—*E.C.R., Jr.*

ADRENALS

CAHILL, G. F.: Pheochromocytomas, *J.A.M.A.* 138: 180 (Sept. 18) 1948.

Four carefully studied cases of pheochromocytoma are described in detail and others referred to briefly. The associated hypertension was sustained in two cases, the B.M.R. was markedly elevated in two cases. Hyperglycemia and glycosuria were not marked in the younger patients. Three cases were in children and one case was associated with Cushing's syndrome. Several of the tumors were extra-adrenal and one was multiple; the intra-adrenal ones were well visualized by x-ray after perirenal air insufflation. All cases showed a drop in blood pressure after the injection of benzodioxane. The danger of operation is lessened by the use of tri-brom-ethanol in anaesthesia induction, by occluding the blood supply to the tumor before handling it, and by the infusion of epinephrine and occasionally adrenal cortical extract after the tumor has been removed.—*A.P.F.*

THORNE, G. W.; FORSHAM, P. H.; PRUNTY, F. T. G., and HILLS, A. G.: A test for adrenal cortical insufficiency, *J.A.M.A.* 137: 1005 (July 17) 1948.

A reliable and sensitive test for adrenal cortical insufficiency is presented. Two striking and easily determined changes (a decrease of 50 or more per cent in circulating eosinophils and a rise in excretion of uric acid) consistently follow the administration of a single dose of adrenocorticotrophic hormone to normal subjects and patients with diseases not involving the adrenal cortex. A decrease of 50 or more per cent in the urinary uric acid-creatinine ratio indicates adequate adrenal cortical reserve. The few conditions in which the test is not useful are described.—*A.P.F.*

FRIEDMAN, S. M.; FRIEDMAN, C. L., and POLLEY, J. R.: Potentiation of the hypertensive effects of desoxycorticosterone acetate by various sodium salts, *Am. J. Physiol.* 153: 226-234, 1948.

The action of sodium chloride in intensifying the effects of DCA on renal function, blood pressure and electrolyte pattern seems specifically referable to the sodium ion. Phosphate may be excessively damaging to the kidney under the influence of DCA. The electrolyte derangement is parallel to the kidney injury. The renal damage caused by salt and DCA is not necessarily accompanied by an increase in hypertension. The increase in blood pressure caused by DCA precedes changes in renal function.—*A.E.M.*

WATERHOUSE, C., and KEUTMANN, E. H.: Kidney function in adrenal insufficiency, *J. Clin. Investigation* 27: 372-379, 1948.

Thirteen patients with adrenal insufficiency were studied with respect to glomerular filtration rate, renal plasma flow and maximum excreting capacity. All patients were in good clinical condition and controlled either on salt alone or with desoxycorticosterone acetate. Treatment, in therapeutic doses, with desoxycorticosterone acetate, testosterone propionate, aqueous adrenal cortical extract, extract of pork adrenal cortex in oil and desiccated thyroid substance was superimposed when possible and renal function studies were carried out. The rates of glomerular filtration were below normal in all patients at all times. Treatment with the hormones resulted in little if any improvement above that found when salt was used in adequate, therapeutic amounts. Large amounts of extract of adrenal cortex resulted in slight increase in filtration rate but the values were still far below normal. The effective renal plasma flow was reduced in all patients except in two cases with a moderate anemia. No change was noted in renal plasma flow by the addition of specific hormonal therapy. The maximum tubular excretory capacity was low in all female patients and normal in all male patients with the exception of two who had hypertension. The authors postulate that the consistent finding of decreased renal blood flow and glomerular filtration rate, regardless of whether or not there is impairment of the ability of the tubular cells to excrete para-amino hippuric acid, is suggestive of a reduction in the effective vascular bed in the kidneys rather than of the level of blood pressure or degree of hydration of the patients. The authors also suggest that the failure of any of the functions to improve with medication may have been due to insufficient dose of adrenal cortex extract, insufficient time of study, and irreversible structural or functional changes in the kidneys. Finally, the factors responsible for maintenance of function mentioned may not have been present in any of the preparations used. The

failure of testosterone propionate to restore maximum excretory capacity in females was felt to be due to the absence from testosterone of the hormonal factors which maintain this function, factors however, that may undoubtedly be supplied by the male testis.—*T.H. McG.*

GONADS

BALZE, FELIPE A. DE LA: The progesterone withdrawal test or "medical curettage." Diagnosis and classification of amenorrheas, *Semana méd.* 55: 953-963, 1948.

The test, originally introduced by Albright, makes use of intramuscular injections of 5 mg. of progesterone daily for five days. Menstruation occurring within five days indicates an adequate estrogen production and mildly subnormal ovarian activity whereas failure to menstruate proves severe ovarian failure. The test was applied to 56 women and compared with the results of vaginal cytological examinations and determinations of gonadotropic hormones in the urine. The test is recommended for its simplicity, reliability and rapidity.—*A.E.M.*

BENDER, S.: The value of the Guterman test in threatened abortion, *J. Obst. & Gynec. Brit. Emp.* 54: 783-792, 1947.

A series of 57 cases clinically characterized as threatened abortion were studied by the Guterman test. This was found to be a rapid, effective, and economical way of distinguishing cases of threatened abortion due to progesterone deficiency, as shown by the urinary pregnanediol excretion. The majority of threatened abortion cases did not show progesterone deficiency; only 11 out of 39 cases showed a negative Guterman test. In 28 patients having a positive Guterman test, the administration of progesterone seemed to have no significant effect in forestalling abortion in the six patients so treated. A possible cause for the threat to pregnancy was found in only six. In a group of 11 showing a negative Guterman test only one of five given progesterone aborted, whereas of the six not so treated only one failed to abort. The authors feel that progesterone given in the absence of progesterone deficiency may favor abortion. They recommend the simultaneous use of the Guterman test and a rapid pregnancy test to help differentiate other conditions from threatened abortion.—*R.A.C.*

FARRIS, E. J.: The prediction of the day of human ovulation by the rat test as confirmed by fifty conceptions, *Am. J. Obst. & Gynec.* 56: 347-352, 1948.

The author studied the time of ovulation in 46 women as determined by reaction of the immature rat's ovary to the hypodermic injection of the urine of the patient. A positive response is hyperemia of the rat's ovaries. Two hundred and eight ovulations were so studied. The lengths of the cycles varied from 24 to 35 days. Conception occurred from cycle day 8 through 19. Sixty per cent of the women conceived on cycle days 11 to 13. The interval between ovulation and the onset of menstruation in the control cycles, during which there was no exposure to pregnancy, ranged from 12 to 20 days, averaging 14.8 days.—*C.D.D.*

HALBRECHT, J.: Further observations on the basal temperature in sterile women, *J. Obst. & Gynec. Brit. Emp.* 54: 848-852, 1947.

From temperatures taken orally or rectally before arising in the morning basal tem-

perature curves were constructed covering over 1100 cycles of normally menstruating sterile women. These curves allow a diagnosis of early pregnancy, delayed menstruation, and early abortion. Desiccated thyroid, progesterone and estrogens do not change the temperature level. The normal rise in the second half of the cycle after ovulation is probably a complex mechanism, the corpus luteum being only one contributory factor. Endometrial biopsies were done in 90 cycles and gave identical results with basal temperature curves in 87 cases. Less than three per cent of all curves were unusable, which is less than the number of biopsies in which definite interpretation is impossible.—*R.A.C.*

KNIGHT, W. R.: Theca-cell tumors of the ovary with a report of 15 cases and a review of the literature, *Am. J. Obst. & Gynec.* 56: 311-324, 1948.

One of the author's patients was studied for the presence of estrone and progesterone in the tumor tissue. The vaginal epithelium showed less than 2+ estrogenic activity as graded by the Shorr method. The biologic assay of the tumor showed 0.2 gamma of estrone in a 100 gram equivalent of the extracted tissue. Pregnandiol assay of a 24-hour urine specimen was negative. The author considers that the estrogen produced by thecoma, although small, may in the absence of progesterone account for the symptoms and frequent occurrence of associated pathology such as menstrual irregularities, post-menopausal bleeding, endometrial hyperplasia, adenomyosis, hypertrophy of the myometrium with uterine enlargement, uterine myoma, and endometrial carcinoma.—*C.D.D.*

KULLANDER, S.: Investigations into the determination of pregnanediol according to the Guterman method, *J. Obst. & Gynec. Brit. Emp.* 55: 159-170, 1948.

A manifestation of Guterman's qualitative method of determining pregnanediol is described. It allows a quantitative determination to be made. Neither the original nor the modified technique permit an accurate diagnosis of pregnancy. Large variations in individual excretion were found during the menstrual cycle and pregnancy. The author says the Guterman test is unreliable in the prognosis of threatened abortion.—*R.A.C.*

LOESER, A. A.: The action of intravenously injected sex hormones and other substances on the blood flow in the human endometrium, *J. Obst. & Gynec. Brit. Emp.* 55: 17-22, 1948.

Using a specially constructed instrument consisting of thermocouples attached to a rubber catheter, changes in blood flow of the endometrium of the human uterus were measured. All hormones tested were injected intravenously, the sex hormones in propylene glycol. Natural estrogens, and to a lesser extent progesterone, produced a vasodilator action. Stilbestrol had only a slight but similar action. Testosterone produced constriction. Adrenalin and pituitrin had strong and lasting constrictor action.—*R.A.C.*

MORISON, C. R.: An investigation of the causes of sterility and lowered fertility in West African Negroes, *J. Obst. & Gynec. Brit. Emp.* 54: 793-816, 1947.

Genital sepsis, chiefly gonorrhoea was the chief cause in 203 cases of sterility studied. Doubt is thrown on the current belief that the menarche starts earlier in African women. Genital maldevelopment and immaturity were found to be more prevalent than in European women. Observations on anovulatory menstruation and endometriosis were made,

though for comparative statistical purposes it is admitted the series is too small. Abortions were found to occur as frequently as in white races whereas uterine myomata, said to be more common in United States Negroes, were not more common in the African group than in whites. There was a virtual absence of psychological factors as a basis for sterility. Reasons are given for this difference.—*R.A.C.*

SEARLE, W. N.; HAINES, M., and BAKER, J. K.: Virilizing tumours of the ovary, *J. Obst. & Gynaec. Brit. Emp.* 55: 135-141, 1948.

A 33-year-old primigravida developed hirsutism, hoarseness of the voice and hypertrophy of the clitoris about the 24th week of pregnancy. Psychologically she seemed normal except for irritability, which she attributed to consciousness of the startling change in her appearance. At the 27th week her blood pressure was found to be 170/120. She was delivered at full term of a normal 5 lb. 6 oz. male child, but failed to lactate. Otherwise the puerperium was normal. A month after confinement a laparotomy was done and an orange-sized cyst on a pedicle was found near the right broad ligament. This did not appear to be an arrhenoblastoma but is adequately described. Improvement in the hirsutism began three months after operation. A few ketosteroid values are recorded. Five years later the patient's appearance and blood pressure were normal.—*R.A.C.*

STALLWORTHY, J.: Facts and fantasy in the study of female infertility, *J. Obst. & Gynaec. Brit. Emp.* 55: 171-180, 1948.

This article reviews the problem of infertility and cites the author's experience. He concludes that the most common cause of unilateral uterotubal blockage is poor coordination of tubal contraction and that uterine irritability is a potent factor in infertility. This may be due to psychological disturbances mediated by the autonomic nervous system as well as to hormonal influences.—*R.A.C.*

TWOMBLY, G. H.; MCCLINTOCK, L., and ENGELMAN, M.: Tissue localization and excretion routes of radioactive dibromestrone, *Am. J. Obst. & Gynec.* 56: 260-288, 1948.

Equilin was brominated with radioactive bromine-82 to produce dibromestrone. This was injected into 12 rabbits, a monkey and 2 dogs. It was found that dibromestrone was not selectively localized in the rabbit in the adrenals, spleen, uterus, ovaries or testes. A high concentration occurred in the gall bladder within 6 hours of injection. Dibromestrone was not selectively localized in the liver, ovaries, or uterus of the monkey or the uterus of the dog. Dibromestrone was weakly estrogenic, if at all. Study was made of the gastro-intestinal tract of rabbits 30 minutes, 2 and 6 hours after intravenous injection. At two hours, 21.5 per cent of the injected dibromestrone was in the small bowel, 4.5 per cent in the cecum, and 0.8 per cent in the large intestine. By six hours, 1.8 per cent was in the small intestine, 12 per cent in the cecum and 10.9 per cent in the large intestine. Solubility tests were consistent with the hypothesis that dibromestrone was excreted into the bile and urine, largely as a phenolic steroid.—*C.D.D.*



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TESTIS-PITUITARY INTERRELATIONSHIP

THE RELATIVE INABILITY OF TESTOSTERONE TO REDUCE URINARY GONADOTROPIN IN EUNUCHOID MEN

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THE following is a report of studies made to compare the effectiveness of testosterone in therapeutic and greater than therapeutic doses with the effectiveness of estrogens, in lowering urinary gonadotropin levels. The subjects were men with severe eunuchoidism.

It is well recognized that high levels of follicle-stimulating hormone are present in the urine of men with testicular deficiency. In the patients studied here this feature has been considered an index of anterior pituitary hyperfunction. Experimentally testosterone has proved capable of preventing histologic as well as physiologic alterations in the pituitary gland of castrate male animals (1). Large doses have usually been employed for such studies. Some clinical evidence has been accumulated which indicates that similar results may, under certain circumstances, be demonstrated in man (2, 3).

The relative inefficiency of testosterone for inhibition of pituitary activity has been shown before. For example Hertz and Meyer demonstrated that in parabiotic rats doses as small as 0.2 gamma of estrone daily inhibit the male pituitary gland as efficiently as 15 gamma of testosterone propionate (4, 5). They also demonstrated that larger doses of androgenic substances are necessary to suppress the pituitary gland in male-female pairs than in female-female pairs. They concluded that the pituitary of the male

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rat was more difficult to suppress than that of the female. The ratio of the two hormones producing comparable effects in parabiotic animals is thus approximately 1 to 75. Catchpole, Hamilton, and Hubert (6) have reported a decrease of the urinary gonadotropin excretion to low levels in man following administration of testosterone propionate.

It has been our experience that in eunuchoid men doses of testosterone considerably greater than needed to overcome clinical evidence of androgen deficiency are necessary to lower the urinary F.S.H. (follicle-stimulating hormone) titer. In our patients the dose of stilbestrol which would lower urinary F.S.H. in eunuchoidism was between 0.5 and 1.5 mg. daily (3.5 to 10.0 mg. per week) and the effective dose of testosterone was in some cases 300 mg. or more per week. Thus our results yielded a ratio between 1:30 and 1:75 in man. Differences in methods of assay may be responsible for the diverse results reported in the literature. Several assays made in our laboratory using the method we employ and an extract made by the method of Freed and Hechter (7) which was used by Catchpole and Hamilton, yielded results much lower than those obtained by means of alcohol-precipitation extracts of the same pooled specimens. If unphysiologic doses of testosterone are necessary to suppress pituitary function to a normal level and physiologic doses fail to do so, a question arises concerning the mechanism by which this balance is normally attained. Is it due to a second testicular hormone (inhibin)? Could it be due to estrogen? It is of considerable interest that estrogens have a much greater power in this regard. On clinical grounds it seems to us somewhat unlikely that estrogens are normally responsible, since the dose found to be necessary to produce such a result in eunuchoid men is similar to the dose which is well known to produce such pronounced abnormalities as gynecomastia when used in men with prostatic cancer.

In the patients reported here testicular deficiency was diagnosed in each case on the basis of physical appearance and the presence of prostatic and genital atrophy and was corroborated by assays for 17-ketosteroids and urinary F.S.H.

Gonadotropin estimations performed in our laboratory on normal subjects by the method described by Klinefelter, Albright, and Griswold (8) yielded values ranging as high as 105 m.u. in twenty-four hours.

Urinary 17-ketosteroid assays were performed on neutral fractions by the MgO adsorption method (9), which, in a series of 48 of our normal male subjects, resulted in values ranging between 6 and 16 mg. per twenty-four hours. Normal levels for our laboratories are shown in the following table. They are given in more detail elsewhere (10).

Estimations performed on the urine of patients whose testes were de-

Normal Levels of Sex Hormone Excretion in Men

Assay	Daily range	Number of assays
17-KS	6-16 mg.	48
F.S.H.	26-105 mouse units	22

ficient showed an increase in the gonadotropin titer in all cases.

Figures 1 and 2 illustrate cases of severe eunuchoidism in which testosterone¹ absorbed from implanted pellets failed to decrease the titer of

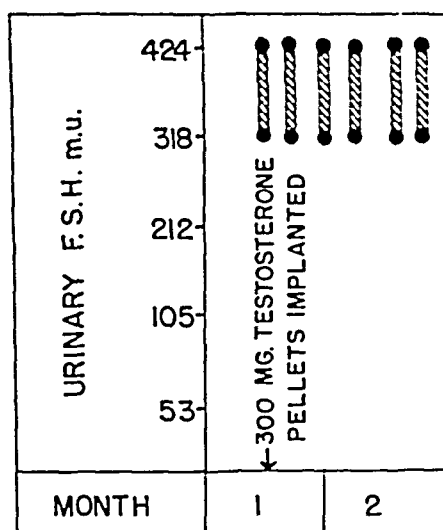


FIG. 1

urinary gonadotropins. In Figure 1, gonadotropin assay prior to implantation of 300 mg. of testosterone yielded a value of 318-424 m.u. in 24-hour specimens. Assays performed weekly for five weeks following the implantation failed to demonstrate any change of this level. In Figure 2, prior to the implantation of 600 mg. of testosterone the F.S.H. level was found to be greater than 105 m.u. on two occasions. The 17-ketosteroid level was estimated in the previous twenty-four hours and yielded a value of 5.9 mg.

¹ The testosterone used in these experiments was generously supplied by the Schering Corporation through the courtesy of Dr. Edward Henderson.

One month following implantation the gonadotropin titer remained unchanged, and the level was again found to be much more than 105 m.u. per twenty-four hours. A third patient with a pre-implantation urinary gonadotropin level of 318-424 m.u. per twenty-four hours was treated by implantation of 600 mg. of testosterone with no resultant depression of the gonadotropin titer according to tests done weekly for four succeeding weeks.

The failure of intramuscular injections of testosterone propionate and aqueous testicular extract to produce a fall in the urinary gonadotropin

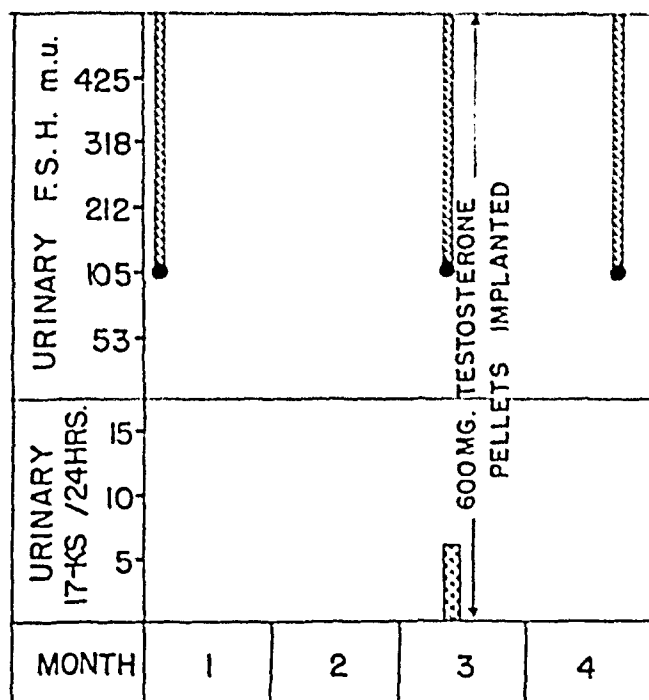


FIG. 2

level is illustrated in Figure 3. Here 100 mg. of testosterone propionate was given intramuscularly each week, and 2 cc. of aqueous testicular extract² was given by injection daily over the periods shown. Assays on three succeeding weeks following the initiation of this therapy yielded titers of 318-424 m.u. of F.S.H. per 24-hour specimen, and four weeks after beginning treatment the titer had risen to 424 m.u. per twenty-four hours. Following discontinuation of aqueous testicular extract the titer gradually fell to 212-318 m.u. per twenty-four hours.

All medication was discontinued for the following twenty-five days, and the titer again reached 424 m.u. per twenty-four hours. The patient was

² This aqueous testicular extract was virtually nonandrogenic and nonestrogenic. It was made from hog testes and obtained through the courtesy of The Upjohn Company, Kalamazoo, Michigan.

then given 3 cc. of aqueous testicular extract daily. In the succeeding three weeks gonadotropin titers remained unchanged, and one week prior to the cessation of aqueous testicular extract therapy, stilbestrol in doses of 1.5 mg. daily was prescribed. Four days later the gonadotropin level dropped

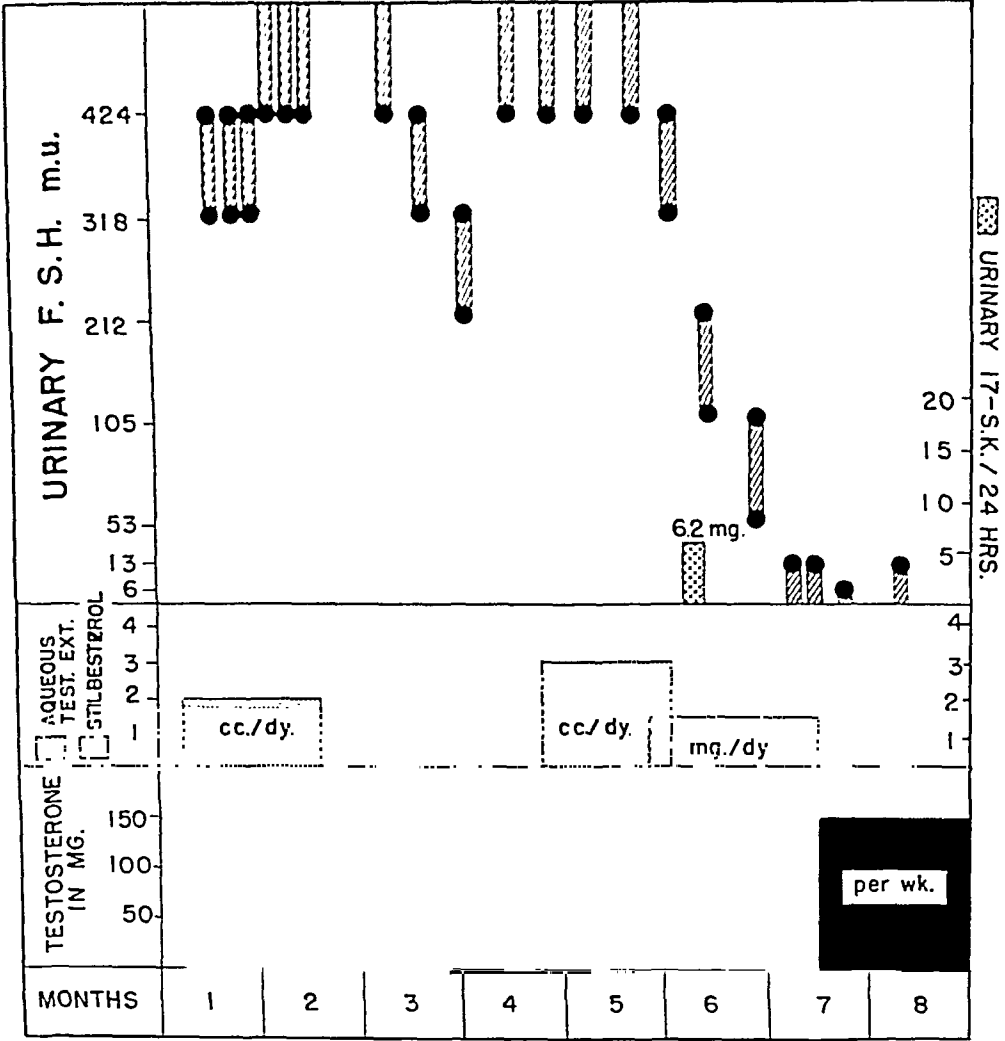


FIG. 3

to 318-424 m.u. One week later during stilbestrol therapy only, the 17-ketosteroid level was reported as 6.2 mg., and the F.S.H. titer gradually decreased, as illustrated in Figure 3, until normal and finally subnormal titers were obtained. Thus testosterone propionate in these doses in conjunction with aqueous testicular extract or the extract alone was shown to

have little effect on gonadotropin excretion. Stilbestrol, in contrast, exerted a pronounced effect on excretion of F.S.H. and presumably an inhibitory action on its pituitary production. The fact that the urinary gonadotropin level remained low for four weeks after cessation of stilbestrol therapy and during therapy with testosterone propionate in doses of 25 mg. six times

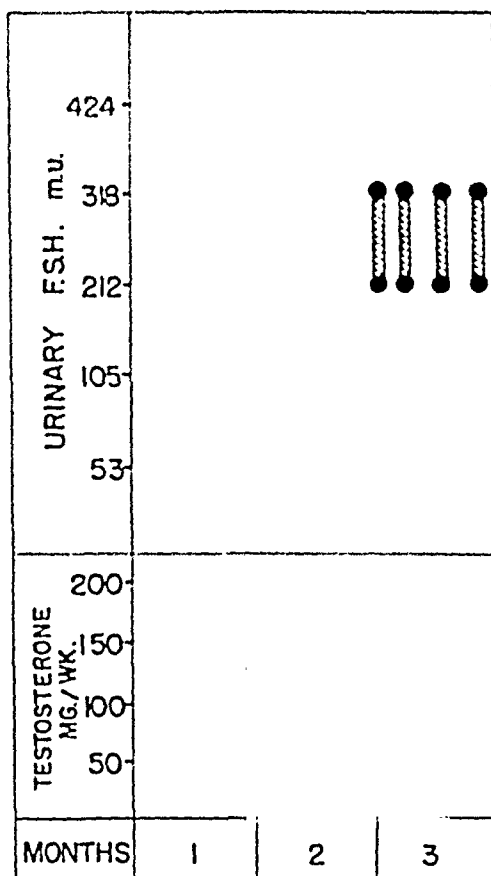


FIG. 4

weekly is, however, consistent with the belief that testosterone may exert a mildly inhibitory effect on the anterior pituitary.

Figure 4 illustrates the failure of the usual therapeutic doses of testosterone to effect the gonadotropin titer. Doses as high as 125 mg. per week for two weeks, 200 mg. for one week, and 100 mg. for five weeks, on repeated urinary F.S.H. estimation, were insufficient to depress gonadotropin excretion below 212 m.u. per twenty-four hours. In Figure 5, a dose of 300 mg. of testosterone propionate per week was used. Prior to the administration of the hormone the gonadotropin level was 212–318 m.u. and the 17-ketosteroid level was 4.7 mg.³ Forty-six days after beginning

³ This assay was made several weeks before the initiation of the above treatment while the patient was receiving intermittent therapy.

therapy with the large doses mentioned, the level was found to be 105-212 m.u. per twenty-four hours. Sixty-three days after institution of this therapy the gonadotropin level reached normal and was reported as 52-105 m.u. per twenty-four hours. Assay of the 17-ketosteroids at this time revealed the high titer of 21 mg. per twenty-four hours, twice the average normal in our laboratory and above normal range. All therapy

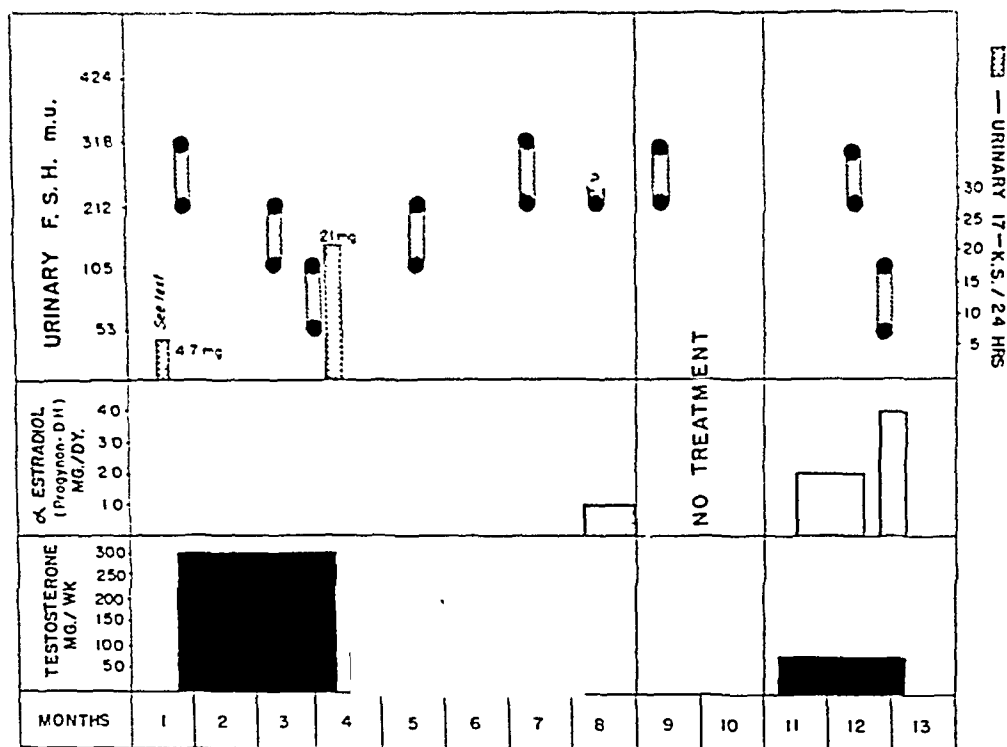


FIG. 5

was then discontinued for one week, and the patient was later given 75 mg. of testosterone propionate weekly. Under the smaller dosage the urinary gonadotropin levels gradually exceeded normal limits once again. These findings are in accord with results obtained by other investigators who demonstrated the depression effect of comparatively massive doses of testosterone on the pituitary gland in experimental castrated animals (11).

All hormonal therapy was discontinued. The patient was then given 0.5 mg. of estradiol orally daily. With this dosage the F.S.H. excretion remained above 212 m.u. per twenty-four hours after twenty-eight days of therapy. The patient then received no therapy for sixty-five days, after which time he again received 75 mg. of testosterone propionate per week.

Eleven days later this therapy was combined with 2 mg. of estradiol orally daily. Two weeks later the gonadotropin level remained 212-318 m.u. per twenty-four hours. Twenty-four days after beginning this treatment the gonadotropin level reached a normal value. Once again the inhibitory action of estrogen upon the anterior pituitary was demonstrated and may

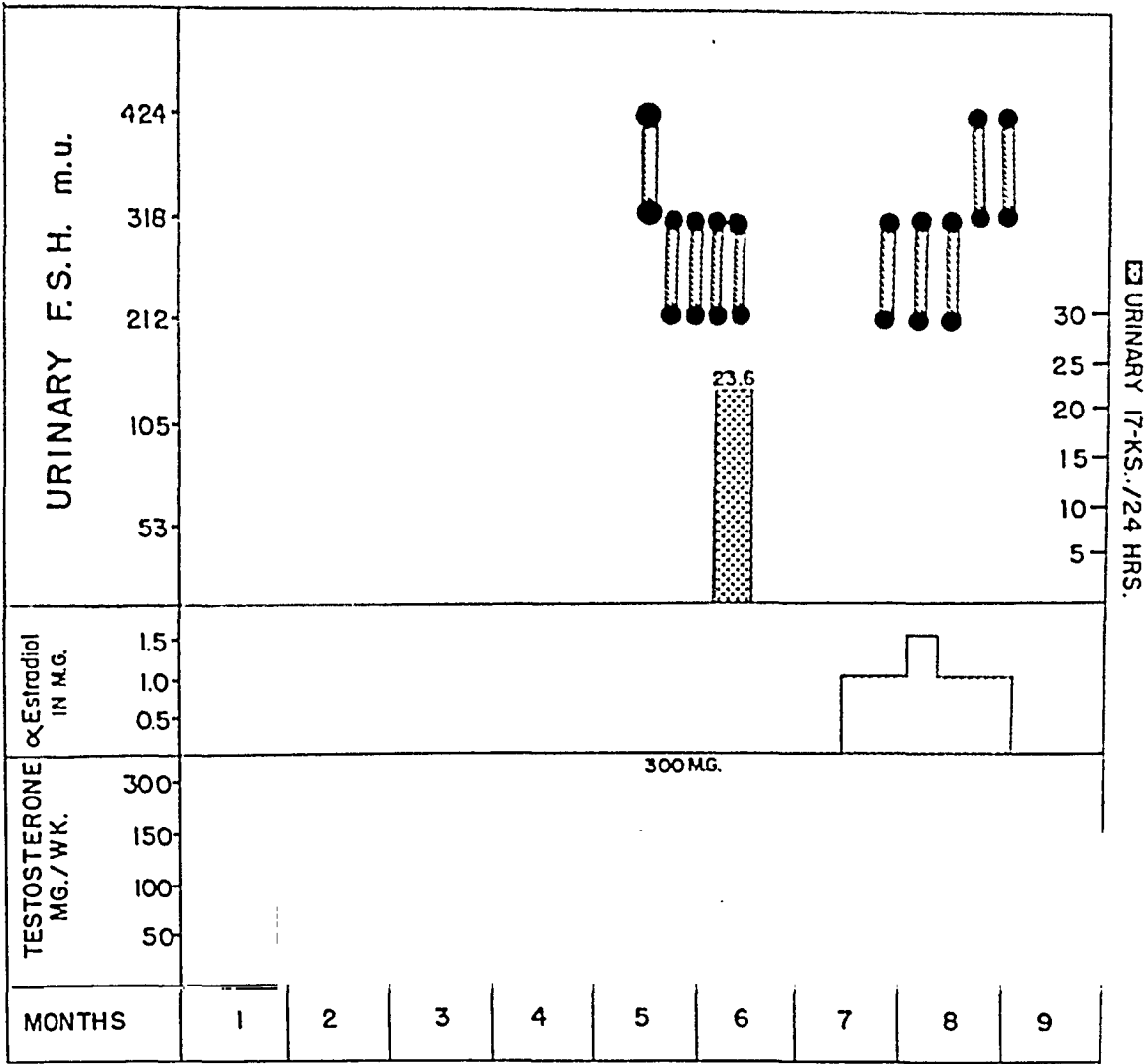


FIG. 6

be compared with similar effects obtained with testosterone in more than physiologic doses. In Figure 6 an instance is shown in which increased doses of testosterone propionate over a period of twelve weeks, culminating in doses of 300 mg. for three weeks, succeeded in depressing the gonadotropin titer slightly. The 17-ketosteroid level at this time was reported as 23.6 mg. per twenty-four hours. The dose of testosterone propionate was then reduced to 150

mg. per week and estradiol in a dose of 1 mg. orally daily was prescribed and later increased to 1.5 mg. orally daily. This did not alter the titer of urinary gonadotropins and gradually the level rose to over 424 m.u. per 24-hour specimen.

In Figure 7 the same severely eunuchoid individual referred to in Figure 6 was given estrogenic substance, having received at least 150 mg. of testosterone propionate per week in the preceding five months. The testosterone propionate and, in addition, aqueous testicular extract in doses as high as 4 cc. per day were incapable of reducing the gonadotropin titer, as evidenced by repeated assays. Stilbestrol was then begun in doses of 0.5 mg. daily with no effect on the F.S.H. level. The 17-ketosteroid level was 13.8 mg., and the gonadotropin titer was reported as over 424 m.u. per twenty-four hours. After seven weeks of therapy the dosage of stilbestrol was increased to 1.5 mg. orally daily, with resultant depression of the F.S.H. titer to 318-424 m.u. per twenty-four hours. Thereupon the dosage of stilbestrol was increased to 2.0 mg. orally daily and the testosterone propionate reduced to 150 mg. per week. During this therapy a greater depression of the gonadotropin level occurred, resulting in normal values. This level was maintained as long as the estrogen was given in this dosage. Reduction in estrogen intake was followed by a gradual elevation of the F.S.H. level, and discontinuation of estrogen therapy resulted in a rise to the original titer of over 424 m.u. per twenty-four hour specimen. After a period of two months during which no therapy was given, the patient was given 100 mg. of testosterone propionate weekly and, in addition received 2 mg. of estrololactone daily. After five weeks of this therapy no noticeable effect was produced, and the gonadotropin excretion remained over 424 m.u. per twenty-four hours.

In Figure 8 are demonstrated the urinary gonadotropin and 17-ketosteroid levels in a eunuchoid individual in whom dosage of 75 mg. of testosterone propionate per week for almost two years had produced a pronounced increase in masculinization. Under this regimen the gonadotropin level still exceeded 212 m.u. per twenty-four hours, and the 17-ketosteroid level was reported as 9.3 mg. per twenty-four hour specimen. An increase in the dose of testosterone propionate to 150 mg. per week for nine weeks resulted in urinary gonadotropin titers of 105-212 m.u. Further increase in the dose of testosterone propionate to 300 mg. per week elevated the 17-ketosteroid level to 12.9 mg. per twenty-four hours but did not alter the F.S.H. level. Stilbestrol in doses of 0.5 mg. daily depressed the gonadotropin level to normal within two weeks. Upon cessation of therapy the titer of urinary gonadotropins gradually increased to the original level of over 424 m.u. per twenty-four hour specimen.

INABILITY OF TESTOSTERONE TO INHIBIT PITUITARY

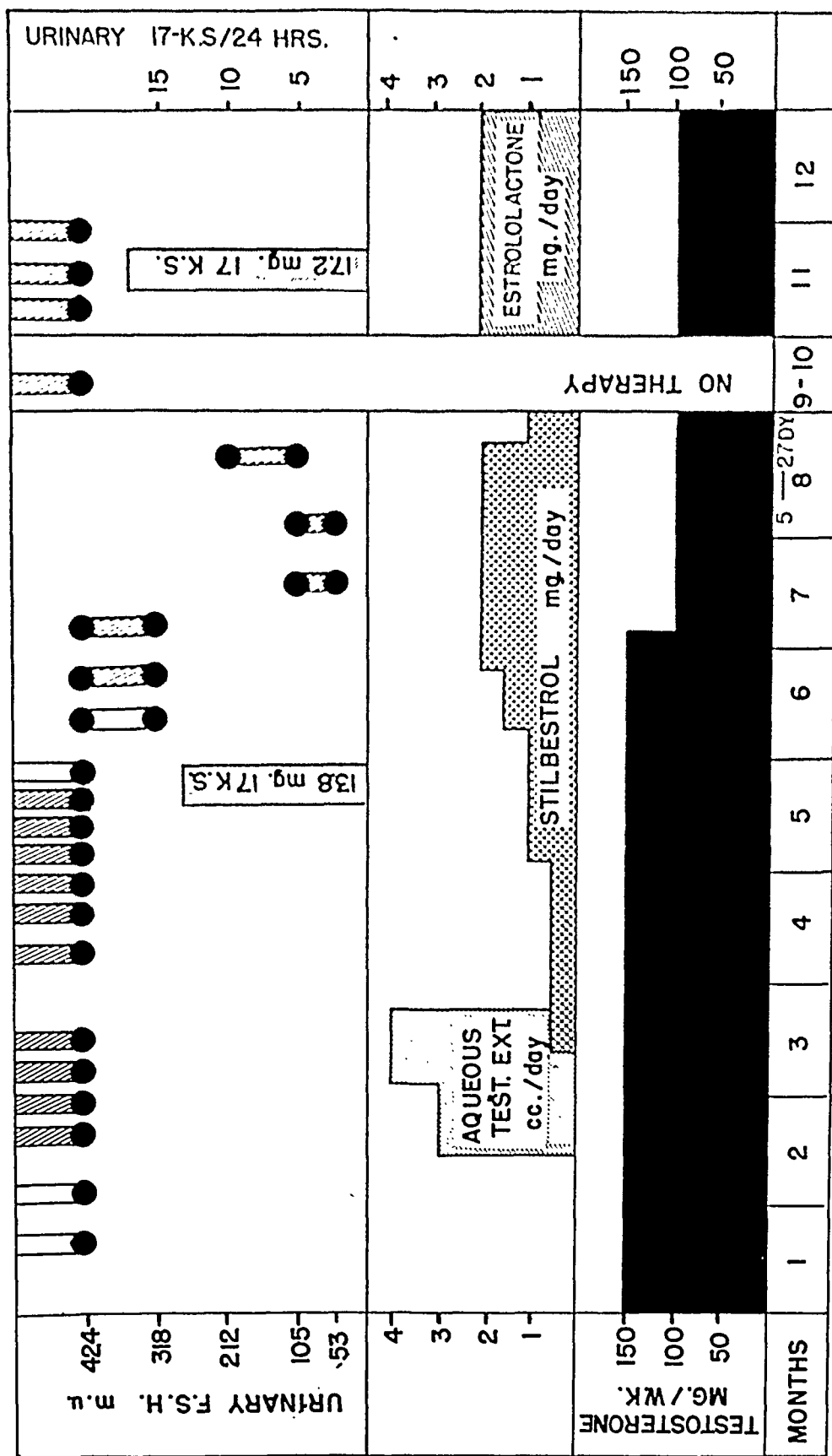


FIG. 7

In the above cases we have demonstrated the relative lack of effect of testosterone on the urinary titer of follicle-stimulating hormone of the anterior pituitary in the urine of patients suffering with testicular deficiency. In the cases illustrated, the use of testosterone in amounts sufficient to

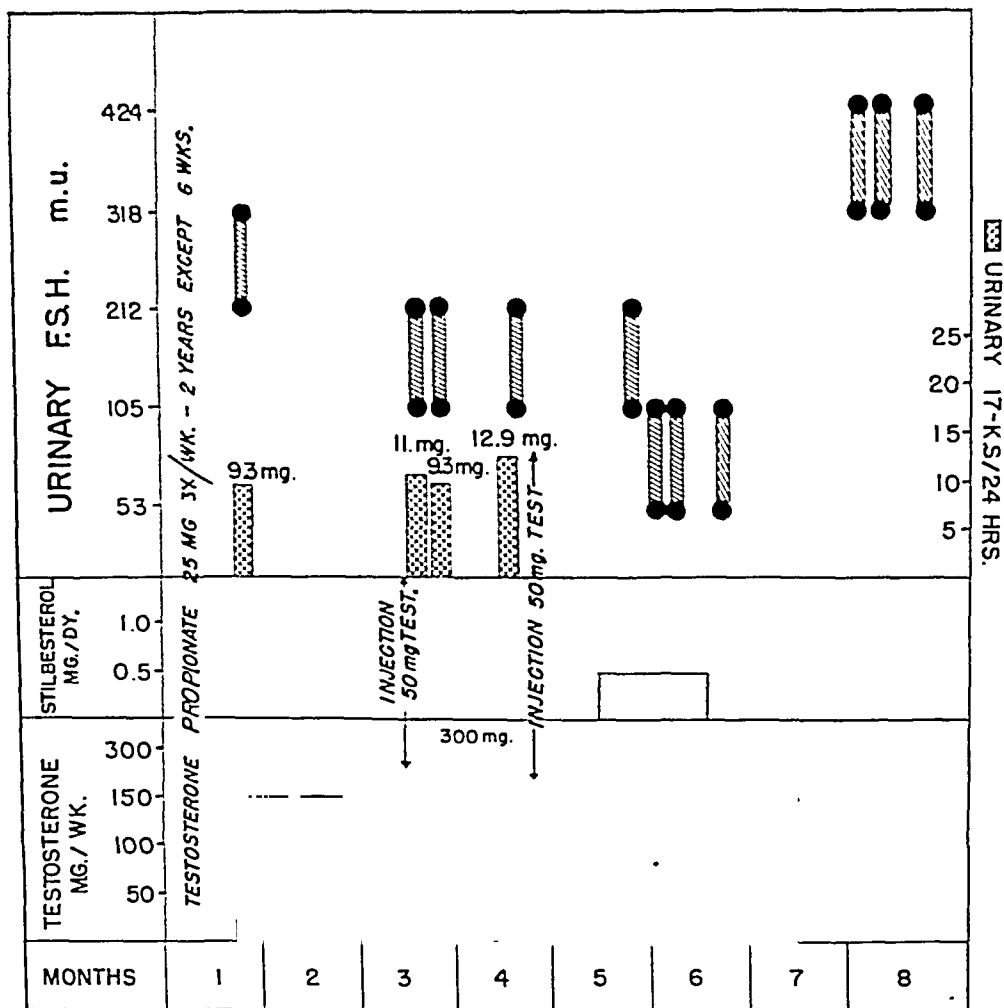


FIG. 8

raise the titer of 17-ketosteroids to normal range asserted little or no effect on the F.S.H. titer. Some influence is shown when large doses were employed. The graphs also demonstrate that estrogens in doses approximating those in common clinical use do lower the urinary F.S.H. in patients with testicular failure.

Although a lack of utilization has been postulated as the reason for high titers of gonadotropins in the urine of eunuchoid men, the reverse could scarcely be an explanation for the lower levels of these substances under the circumstances cited above. In these instances pituitary suppression would appear a more logical explanation.

CONCLUSIONS

1. In cases of severe hypogonadism in the male, urinary F.S.H. titers were not reduced to normal levels by administration of testosterone in the usual therapeutic form and dosage, and in amounts sufficient to create normal titers of urinary 17-ketosteroids and clinical signs of masculinization. Very large doses of testosterone apparently do have this power, as has been previously claimed.

2. Stilbestrol when employed in ordinary therapeutic doses does lower the urinary F.S.H. titer in primary hypogonadism in men.

3. Estrololactone in doses of 2.0 mg. per day failed to lower urinary gonadotropins in one patient.

4. Testosterone in the doses employed here is adequate to overcome androgen deficiency, but fails to inhibit pituitary activity. These facts are consistent with the theory that a second testicular hormone (inhibin) exists normally, and that it has a pituitary-inhibiting power similar to that of estrogen.

Acknowledgment

We wish to thank Miss D. C. Tweed and Miss B. J. Sipher for the careful technical assistance which made these studies possible.

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THE TREATMENT OF ACROMEGALY

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THE development of bony and tissue changes in patients with acromegaly is so insidious that the victims of this disorder as a rule seek medical assistance only in its advanced stages. Very little can then be accomplished in the correction of these effects.

On the other hand, satisfactory treatment of acromegaly is usually possible providing the condition is recognized early, and if energetic attempts are made to follow patients with this malady at regular intervals. The unpredictable course of acromegaly, however, renders evaluation of treatment difficult in some cases. This review of our experience in the management of acromegaly was undertaken to evaluate past treatment with the hope of possible improvement in the future.

The first objective of treatment is the relief of symptoms resulting from the expanding tumor, if such exists, which may cause headache and visual or other neurologic disturbances. Many patients with acromegaly are not affected in this way, so that the question of treatment of secondary hormonal signs or symptoms must be investigated. Occasionally an enlarged sella is found on roentgenologic studies of an individual without obvious acromegaly and lacking symptoms or evidence of pressure effects, and in whom there is no evidence of hormonal change even after exhaustive analyses. Since early treatment of this condition is important, such cases must be included as subjects for therapy whenever any change indicative of expanding tumor or increased hormonal activity becomes apparent. At this time as well as on first examination we are confronted with the problem of demonstrating active acromegaly or hyperpituitarism. Signs or symptoms of increased local pressure from the tumor do not necessarily mean hormonal overactivity, but an attempt should be made to relieve pressure regardless of the question of progressive acromegaly. Amenorrhea in the female and impotence in the male may usually be considered secondary effects due to pressure, since these symptoms occur chiefly after considerable enlargement of the sella. These dysfunctions are sufficient to warrant conservative therapy, namely radiation.

If all evidence of intracranial and intrasellar pressure is absent, how can one be sure that hyperactivity is nonexistent? The presence of acromegalic

changes, *i.e.*, prognathism, coarsened facial features, enlargement of the tongue, hands and feet, does not indicate activity at the time of the initial examination, and since these findings develop so insidiously, months of observation may be necessary to establish the presence of hypersecretion of the pituitary growth factor. Two other hormonal effects are subject to clinical appraisal; namely, the thyrotropic and the diabetogenic factors. The first usually causes enlargement of the thyroid and an elevated basal metabolic rate together with clinical hyperthyroidism. Active diabetes, particularly of recent origin, is often considered evidence of excess pituitary secretion, yet it is our impression that either or both of these hormonal complications may be present without concomitant progressive enlargement of the acral parts. Explanation of the first phenomenon might be found in the catabolic nature of hyperthyroidism offsetting the anabolic nature of early active acromegaly; and in the second instance, as suggested by Young (1), diabetes may be the result of exhaustion of the islets of Langerhans following prolonged overstimulation.

Reifenstein (2) *et al.* have suggested that the level of blood phosphorus, because of its elevation during the active growth period in a normal individual, may be used as an index of growth hormone in acromegaly. These authors apparently consider 3.2 mg. per 100 cc. as the average normal value. In observations on 19 cases, 4 of which were from this series, we obtained values as shown in Table 1. There appears to be a lowering in these cases after roentgen therapy. Persistent values over 4.5 mg. per 100 cc. point to increased growth activity, but we believe that too much emphasis should not be placed on single estimations, especially between values of 4 to 4.5 mg. per 100 cc., because of the possible inherent error in the analysis when carried out in routine clinical laboratories. Specimens should be taken during fasting, since food tends to lower blood phosphorus.

The term "fugitive" acromegaly, used by the late Dr. Cushing, is in itself a fugitive term and unless the individual in question has an enlargement of the sella or some other stigmata of previous pituitary hyperactivity, such a diagnosis is largely conjecture. The condition is most frequently seen in cases of chromophobe or mixed pituitary tumor. Sosman has pointed out that cases of chromophobe tumor may have a brief period of pituitary hyperactivity with slight acromegalic changes, followed soon by the classical chromophobe signs and symptoms.

In young females, when evidence of pressure is lacking and when there are in the face and extremities early tissue changes which may be partially reversible together with a minimum of irreversible bony enlargement, early therapy is justified even without the usual clear-cut evidence of hyperactivity. In these cases particularly, a persistently high blood phosphorus should be helpful.

TABLE 1. BLOOD PHOSPHORUS DETERMINATIONS IN ACROMEGALY

Serum phosphorus, mg. per 100 cc.

Considered active clinically			Considered inactive clinically		
Case No.	Before treatment	After treatment†	Case No.	Before treatment	After treatment
1	5.1	4.2	13		3.4*
	5.5		14	3.9	4.0
2	5.0	4.5	15	4.4	
	4.7	4.4	16		3.2**
		3.7	17		3.8**
3	5.0	4.1			3.2
	4.1	3.2	18		3.1‡
4	4.4		19		4.3
	3.5				
5	4.5	3.5**			
6	4.1				
7	4.0				
8	4.1				
9	4.2	4.1			
	4.1	3.5			
10	5.1	4.7§			
	4.9	3.9			
		4.1			
11	4.4	3.1			
12	4.4	2.8			

Average before initial treatment, active cases, 4.52.

Average after initial treatment, active cases, 3.9.

Average, inactive cases, 3.5.

† Treatment usually consisted of one course of x-ray therapy between first and second figures. Subsequent figures listed under "Before treatment" show values before second course, in patients still being treated.

* "Burned out." No treatment.

** After surgery and x-ray treatment.

‡ After x-ray treatment.

§ We are indebted to Dr. Anna Forbes of the Massachusetts General Hospital for some of these analyses in this case.

One must rely upon the history in some patients to establish early acromegalic effects since prognathism may not be detectable; but if suspected, enlargement of the hands and feet must also be present to render questionable prognathism of significance. Radiation therapy, in these cases, too, is the logical treatment for at least six months or a year unless there is in-

creasing impairment of the visual fields. During this time an attempt may be made to find evidence of progression by careful comparative measurements of the sella turcica, photographs, roentgenograms or plaster replicas of the face and hands. The volume of water displaced by hands was used many years ago by Faltz (3) for estimating tissue changes. In young females it is desirable, if not imperative, to preserve normal facial features, and surgical removal of the tumor should be seriously considered as the most logical therapy if no other contraindications exist. It must be remembered, however, that surgical removal of the tumor is not always satisfactory and carries a certain risk. While it may thwart acromegaly, there is no positive assurance that the adenoma will not recur because of technical difficulties in its enucleation. Furthermore, if a young acromegalic female is menstruating regularly and wishes children, either radiation or surgical treatment might be followed by amenorrhea, although we have not seen this happen after either form of therapy in such cases. Both types of treatment may restore normal menstruation (and ovulation?) in the amenorrheic, acromegalic patient, but there is a possibility of permanent cessation of the catamenia after surgical removal of the adenoma. The decision as to which course to pursue in such cases is difficult, but the safer certainly is roentgen therapy, if any treatment at all seems warranted before conception.

Objectives of treatment in acromegaly may be summarized as follows:

A. Relief of pressure and its effects

1. Relief from local pressure symptoms on adjacent structures
 - a. Headaches.
 - b. Visual disturbances (*i.e.*, field defects and impaired acuity).
 - c. Prevention of extension of growth into the sphenoid sinuses or above the sella intracranially, and possibly recession of invasion in these areas.
 - d. Mental or neurologic changes, such as convulsions, hemiplegias and psychoses.
2. Relief from pressure within the sella by the tumor on normal pituitary tissue or on the neurohypophysis.
 - a. Amenorrhea in the female.
 - b. Impotence in the male.

B. Relief of hyperhormonal effects

1. Prevention of further bone changes or diminution of tissue effects of acromegaly with possible restoration to normal in early cases.
2. Relief of headaches (which may be of hormonal origin).
3. Relief of hyperthyroidism.

4. Control of diabetes.
5. Decrease in cardiovascular symptoms, such as hypertension or congestive heart failure.
6. Relief of symptoms from bone damage, such as hypertrophic arthritis, exostoses, kyphosis or other orthopedic complications; and perhaps prevention of these progressive conditions.

C. Relief of late or concomitant hypohormonal effects

ROENTGEN DIAGNOSIS

The roentgen diagnosis of chromophil tumors is dependent upon the findings in the cranial vault, and the most important change is the direct pressure effect upon the sella turcica. In a tumor arising within the sella itself and held in check superiorly by the capsule, there is a gradual ballooning of the sella, producing thinning of the bony wall, depression of the floor into the sphenoid sinuses, dorsal displacement of the dorsal clinoids associated with thinning and occasionally fracture, or complete absorption. The anterior clinoids may be pushed farther apart than normal. Unilateral enlargement is not uncommon. The size of the sella varies in normal individuals and it is necessary, therefore, to establish its limits arbitrarily; thus, a tumor may be of considerable size, having produced bone and soft tissue changes, before the sella becomes enlarged beyond the upper limits of normal. We believe a sella turcica 12 mm. deep by 15 mm. wide is a satisfactory standard for the upper limits of normal, and 22 to 26 mm. as the normal dimension for the distance between the anterior clinoids when measured in the postero-anterior view.

For some time we have been using the lateral contour area, as measured in square millimeters on transparent ruled paper, after the method of Haas, (4), for successive serial comparisons of sellar size. We believe this probably provides a more accurate method than using depth and width measurements of the sella turcica for such purposes. The average area of the lateral contour is approximately 74 sq. mm. with a normal range of from 70 to 130 sq. mm. Values of 130 to 150 sq. mm. may be considered borderline. In the presence of other findings, such as thinning of the floor of the sella, and enlargement of the accessory sinuses or prognathism, the diagnosis should be suggested regardless of the size of the pituitary fossa.

CLINICAL MATERIALS AND METHODS

Twenty-nine patients with acromegaly were seen at the Lahey Clinic from 1932 to 1945 (Tables 2 to 5). Clinical data on these cases for the most part have been described elsewhere (5). Of the 29 patients, 1 was untraced, 7 were reached by mail, and 6 are dead. Of these, 20 patients re-

ceived at the Clinic either radiation or radiation and surgical treatment directed toward the pituitary itself, as follows: surgical removal of the pituitary tumor, 5 cases (followed by radiation in 2 cases); surgical exploration without removal of tumor, 1 case; and roentgen therapy without surgical removal of the adenoma, 14 cases. Three other patients in this series received radiation therapy elsewhere. In addition, other therapeutic procedures of importance were: subtotal thyroidectomy, 2 cases; thiouracil for hyperthyroidism followed by subtotal thyroidectomy, 1 case; removal of osteoma of the orbit, 1 case; treatment to relieve pain of dorsal kyphosis, 2 cases; and for pain of hypertrophic and mild arthritis, 2 cases; stilbestrol administration in an attempt to relieve headache and hot flashes, 2 cases; for persistent lactation, 1 case; and for amenorrhea, 1 case.

Method for Radiation Therapy

Radiation is generally considered the method of choice in the treatment of chromophil pituitary adenomas producing acromegaly (6) (Table 2). Marked visual-field defects are not frequent in this disorder so that in most instances radiation is used in cases of headache and to decrease the size of the tumor in an attempt to relieve the compressed portions of the normal gland and thus permit it to function again, as well as to decrease the hormonal secretion of the tumor itself.

No uniform plan of roentgen dosage was employed; treatment was given empirically when deemed necessary because of symptoms. A review of the therapeutic results in this group shows that those who received larger doses of irradiation have had, as a rule, greater and more lasting relief. The lethal dose for this tumor is not known, and no patient has received sufficient radiation to produce clinical evidence of brain or bone damage and no severe skin changes have thus far developed.

The present plan of radiation therapy is to direct the beam through two lateral portals using 200 k.v.p., 20 ma., 50 cm. distance, 1 mm. of copper plus 1 mm. of aluminum, HVL 3.7 mm. copper, giving 300 to 400 r (measured in air) daily through a 5 cm. portal, treating one portal daily, with the x-ray beam directed through the temporal region toward the sella turcica. A series of ten treatments with the total dose of 2000 r to each temporal region, is given. The approximate tumor dose delivered to the pituitary was 50 per cent of the air dose or a total tumor dose of 2000 r delivered to the tumor. This may be repeated eight weeks later, although if the tumor is radiosensitive an initial series of ten treatments should be large enough to produce good palliative results. If relief is not attained by this amount of therapy, it may be because the lesion is radioresistant and possibly cystic. It is fair to assume, however, that more persistent pal-

TABLE 2. ACROMEGALY TREATED BY ROENTGEN RAYS

Case No., age (yrs) and sex	Duration of disease	Duration of observation	Degree of acromegaly	Headache	Headache relieved	Amenorrhea	Amenorrhea relieved	Visual field defect	Visual field defect relieved	Roentgen ray therapy, No. of treatments	Duration of roentgen ray therapy	Duration of follow-up since last treatment
1 27 M	5 yrs.	11 yrs.	III	+	+	—	—	0	—	1930 8 1932 8 1934 6 1935 10	13 yrs.	1 yr
2 36 M	8 yrs.	8 yrs.	II	+	+	—	—	0	—	13	2 yrs.	8 yrs.
3 30 F	1 yr.	7 yrs.	II	+	+	0	—	—	—	42	6 yrs.	1 yr
4 50 F	10 yrs.	4 yrs.	I	+	+	—	—	+	+	26	2 yrs.	—
5 43 F	2 yrs.	7 yrs.	I	0	—	0	—	0	—	2	2 days	None
6 48 F	9 yrs.	6 yrs.	II	+	+	—	—	—	—	24	3 yrs.	4 yrs.
7 20 M	5 yrs.	3 yrs.	II	0	0	0	0	Appeared 1 yr after first visit	—	—	2 yrs.	Under treatment.
8 32 F	2 yrs.	5 yrs.	I	+	0	+	0	0	0	36	4 yrs.	1 yr.
9 46 F	6 yrs.	5 yrs.	II	+	+	+	+	0	—	24	6 mos.	18 mos. 36 mos.
10 32 F	2 yrs.	16 mos.	I	0	0	+	+	0	—	12	1 yr.	9 mos.
11 41 F	4-12 yrs.	11 yrs.	II	+	0	+	0	0	—	9	6 mos.	10 yrs.
12 40 M	10-18 yrs.	+	II	+	+	—	—	0	—	12	6 mos.	3 yrs.
13 37 F	6 yrs.	1 yr.		+	+	Excess and irregular	Became regular	0	—	10	6 mos.	None after last x-ray treatment
14 37 M	5 yrs.	3 mos.	IV	0	0	0	0	++	++	12	3 mos.	Under treatment

liation may be obtained by further treatment, a course we are now pursuing.

It is customary, following the plan of Sosman, to give irradiation usually within the first month, to all postoperative cases. This treatment consists of the same factors as previously outlined.

TABLE 2—(continued)

Sella size before treatment (mm.)	Sella size after treatment (mm.)	Other data	Other treatment	Comment
16×19 (1930)	15×21 14×19 (1944)	Tinnitus	None	No progress in acromegaly. Some changes in temperament. Libido excessive. General condition good.
28×35	21×35	Protrusion of tumor into nasopharynx		Complete erosion of sphenoid sinus and protrusion into nasopharynx. Receded completely after x-ray therapy. Tissue regression noted. No symptoms of progression of acromegaly. Considered stationary.
14×17	13×15 ? well formed		One testosterone pellet	No advance in acromegaly. Temporary relief of headache after x-ray therapy. Amenorrhea after first series of x-ray treatment proved to be due to pregnancy. Delivered normal child without incident.
21×26		BMR +43; diabetes	40-6-15 units of insulin	Natural menopause. Headaches and visual field defects relieved. BMR fell to +7. Died in diabetic coma after stopping insulin. Tissue regression noted.
17×12 (1936)	19×14 (1943)	BMR +20; goiter	Subtotal thyroidectomy in 1936	Operated on for goiter in 1936 at which time acromegaly was recognized. Sella was not enlarged. Seen again in 1943. Sella enlarged. Patient became psychotic after 2 x-ray treatments. Died in state hospital of acute infection in 1943.
14×15	11×13		Benzedrine, stilbestrol 5 mg. weekly	Natural menopause at 42. Relief of headache for 4 yrs.; returned. Fatigue came on after x-ray therapy. No help from stilbestrol 5 mg. parenterally weekly. No progress in acromegaly.
R15×23 L22×33	R22×25 L30×34 (See Figs. 1 and 2)	Severe acne	Estrone sulfate or stilbestrol up to 15 mg. daily	Acromegalic gigantism; reduced libido. Testes somewhat atrophic. No progress of acromegaly for 2 yrs., then reduced vision and enlarged sella. Surgery in 1946.
20×21	21×22 23×25	Persistent lactation	Stilbestrol, 5 mg. weekly	Onset of acromegaly after pregnancy. No relief of headache or slowing of lactation with x-ray. Some increase in acromegaly. Temporary relief of headaches and lactation with stilbestrol. Increasing size of sella.
15×16	15×18 14×18		Stilbestrol, orally, 0.5 mg. daily	Unilateral enlargement of sella. Improvement in headaches but not complete. Periods returned, but irregular. Hot flashes controlled by stilbestrol.
10×15	12×15			No progress in acromegaly. Improvement in acne. Periods returned.
2 times normal				Headaches persist. No other change. Degree of acromegaly not known but patient writes "condition same."
21×22	19×22	Marked hyperthyroidism; goiter	Iodine; thiouracil	Marked sweating, tremor, tachycardia. BMR +70. No help with x-ray or iodine. BMR +11 on thiouracil. Subtotal thyroidectomy; good result for 3 years, then recurrence.
Films lost				X-ray treatment seemed to stop progress which had been steady for six years. Some tissue regression noted. Excessive flow stopped, as did much of headache. No follow-up after last x-ray treatment. Epileptic attacks (several) which did not recur for 1 yr. after treatment.
20×22 Depressed into sphenoid		Obliteration of oral pharynx by tongue		Chronic exhaustion; excessive drowsiness. Thyroid normal; BMR +5. Positive glucose tolerance curve. Response to 12×300 r. Excellent symptomatic relief.

RESULTS

RADIATION THERAPY

1. Tissue changes

As a result of radiation, regression of the enlarged soft tissues may take

TABLE 3. ACROMEGALY TREATED BY SURGERY AND IRRADIATION
(up to 1945)

Case, No., age (yrs) and sex	Duration of disease on admission	Duration of observation	Degree of acromegaly	Headache	Headache relieved	Anosmia	Anosmia relieved	Visual field defect	Visual field defect relieved	Surgery of tumor	Other surgery	X-ray therapy, No. of treatments	Duration of x-ray therapy	Duration of follow-up since last therapy
15 35 F	8 yrs.	6 yrs.	II	+	0	+	+	0	+	1942 1947 1940	0	40	12 yrs.	-----
16 30 M	3 yrs.	9 mos.	II	++	0	-	-	Slight edema	-	Not removed; explored	0	2 series	3 mos.	3 mos.
17 23 F	11 mos.	1 yr.	I	+	+	+	0	++	+	Yes	0	6 p.o.	p.o., 1 wk.	12 mos., 40 mos.
18 28 F	3 yrs.	15 mos.	II	++	+	+	0	0	0	Yes	0	12 p.o.	3 mos.	18 mos.
19 33 F	8 yrs.	5 yrs.	II	+	0	+	+	?	0	Yes	0	18 before op., 6 after op.	6 mos.	24 mos.
20 48 F	5 yrs.	1 yr.	III	+	+	+	0	++	+	Yes		7 before op., 8 after op.	2 mos.	9 mos.

place. These changes are often noted by the patient; namely, that the tongue feels and becomes smaller, and the flesh of the hands, particularly of the palms becomes more pliable. These results can be expected in four to eight weeks following radiation. Attempts to demonstrate this change by photographs and plaster casts as yet have not been satisfactory, but it should be possible to illustrate tissue regression in some cases; however, these procedures have not been used extensively. As far as we have been able to tell, only 2 of our treated patients have shown any noticeable progress in the enlargement of the acral parts after treatment was begun (Cases 7 and 8).

2. Visual changes

In one case visual field defects were completely eliminated following radiation. Improvement of visual acuity was observed in another patient with generally restricted fields. Two other patients were thought to have slight improvement in minor visual field changes, but this was equivocal. Marked improvement in visual field defects occurred in two other patients but both have been subsequently operated upon (1946), one because of a sudden hemorrhage within the adenoma some months after the last course of radiation (Case 20), with great visual impairment, and the other,

TABLE 3—(continued)

Pathologic report of tumor	Sella; size (mm.)	Sella; size after treatment (mm.)	Other data	Results of treatment
Mixed, acidophilic predominantly	Enlarged	?	X-ray therapy before surgery failed to relieve. Associated attacks of unconsciousness; mental upsets.	Much improved by first operation 1932. Second operation helped—extensive recurrence. Third operation performed elsewhere when patient was moribund. Dead. X-ray therapy did not help headache. Died 9 mos. later of leukemia. Blood examination before treatment gave normal results.
Mixed, with hemorrhage	18 X 19	15 X 19 to 19 X 20	Stilbestrol orally for six months, 0.5 mg. daily	No progression of acromegaly. Still amenorrheic and complains of fatigue. Good tissue regression. Recurrence 42 months later; larger sella. Controlled by x-ray.
Specimen deteriorated	17 X 20	16 X 17 1 yr. recalcification	Hyperthyroidism present. BMR +44	No progress of acromegaly. Gain of 50 pounds. Hyperthyroidism improved. Good tissue regression.
Eosinophilic	14 X 21	15 X 20	Received stilbestrol propionate 5 mg. weekly for several months with no help.	18 x-ray treatments given in period of 6 mos. failed to relieve headache or amenorrhea permanently. Tumor progressed in size in 2 yr. period following. No progress in acromegaly. Surgery caused tissue regression, and relief of amenorrhea. Headaches continue but less severe.
Unclassified	20 X 11	21 X 10	Amenorrhea due to hysterectomy	Halved vision in one eye before irradiation, improving at first on x-ray therapy, then sudden, rapid progression. Complete restoration of visual field following surgery.

because of an enlarging sella and decreasing vision in spite of radiation (Case 7)—see section on treatment with estrogens (Figs. 1 and 3).

3. Changes in the size of the sella turcica (Table 5)

In 14 cases we were able to make roentgenograms of the skull from time to time, and the following observations were made. In one case (Case 5) in which the diagnosis of acromegaly was made in 1936, a subtotal thyroidectomy was successfully performed because of hyperthyroidism. At that time the sella was not considered to be enlarged. The patient returned seven years later because of headache. The sella had increased from 17 by 12 mm. to 19 by 14 mm. Two roentgen treatments were followed by acute psychosis. This patient was transferred to a mental hospital where she died subsequently of pneumonia, never having recovered from her psychotic state. She should have returned for periodic examination, since earlier radiation therapy might well have prevented the expanding tumor and probably the psychosis. In another acromegalic (Case 7; Figs. 2 and 3), no progressive enlargement was detected during the first year of observation, but it rapidly developed in the second year. No treatment was given in this case until visual field defects as well as a change in the acromegaly was noted. In 4 other cases in which inadequate therapy was given

TABLE 4. PATIENTS WITH ACROMEGALY IN WHOM THE PITUITARY WAS NOT TREATED AT THIS CLINIC

Case No., age (yrs.) and sex	Duration of disease	Duration of observation (up to 1945)	Degree of acromegaly	Headache relieved	Headache	Return of amenorrhea	Visual field defect	Surgery	Size of sella (mm.)	Other data	Comments
21 34 F	8 yrs.	6 yrs.	II	+	0	0	0	Subtotal thyroidectomy. BMR ± 55	18 X 18; enlarged; depressed into sphenoid sinus	Marked hypertrophic arthritis with dorsal kyphosis	Good result from operation. Bact. deformity still disabling. No x-ray treatment; <i>fluvi-viva</i> far away. Not known if acromegaly progressed. Follow-up during first year.
22 58 M	Long time	2 mos.	III	-	0	-	0	BMR ± 57	Enlarged; clinoids separated 11 X 24	Destructive lesion of D6 and D7. Hypertrophic arthritis marked.	Transferred elsewhere; died of subacute bacterial endocarditis. Thyroid moderately enlarged. No response to iodine clinically.
23 40 M	25 yrs.	6 mos.	II	0	-	-	0	Removal of osteoma of orbit 3.4 X 4.5	19 X 20	Incomplete fusion right hip	Successful removal of osteoma of orbit which produced protrusion of left eye.
24 41 F	11 yrs.	7 yrs.	III	?	-	+	0	Radical left maxillary sinus operation	Enlarged sella eroded down to sphenoid sinus	Cervical, dorsal kyphosis	Marked increase of acromegaly according to patient's letters. No other treatment carried out. Patient lived in northern Canada. Not possible to be treated at home.
25 63 M	?	1 exam.	II	0	0	-	0	0	Enlarged, 15 X 21		No complaints. Unaware of condition.
26 50 M	5 yrs.	11 yrs.	I	0	-	-	0	0	Moderate, 13 X 13	Paravertebral, coronary sclerosis; hypertrophic arthritis	Patient died in 1944.
27 20 F	2 yrs.	1 visit	II	?	+	?	-	0	Enlarged; erosion of clinoids		X-ray advised. Treated elsewhere; no follow-up. Shoes changed from 5 1/2 to 7 1/4, then remained stationary.
28 47 F	3 yrs.	8 yrs.	I	+	0	0	0	0	Enlarged; floor depressed	Diabetes	X-ray; 13 treatments elsewhere. Follow-up says "continued headache." Afraid to have more treatment. Wears half size larger shoes. High blood pressure.
29 29 F	4 yrs.	+	II	0	-	+	0	0	6 X 9		X-ray begun elsewhere. Headaches relieved with x-ray. Not observed long enough for conclusions. Diabetes mellitus.

BONES (cont.)

- study of function, with radioautography, 1153*
- ossification of, in precocious puberty associated with ovarian dysgerminoma, 1349*
- osteitis deformans; glucose tolerance curves in, 907*
- osteitis deformans; insulin for relief of pain in, 385*
- osteolytic metastases; hypercalcemic syndrome associated with sex hormone therapy in, 1*
- radioiodine content of red marrow and of metastases, 56 hours after oral ingestion of I^{131} for thyroid cancer, 1379*

BOOKS RECEIVED, 474

BRAIN: *see also* Emotion; Mental disease; Psychoneurosis; Nervous system; Pituitary

- decerebrate rats; carbohydrate metabolism in 670†
- electrocerebral dysfunction and spontaneous hypocalcemia, 398
- electroencephalogram; effect of Compound E on, in Addison's disease, 660†
- electroencephalogram in Turner's syndrome in the male, 1333*
- encephalogram in pheochromocytoma with hypothalamic manifestations, 782*

BREAST

- cancer; androgenic and estrogenic therapy of; hypercalcemic syndrome associated with, 1*
- carcinoma, inoperable; effects of testosterone on peripheral blood and on bone marrow in, 666†
- carcinoma; metastases to bone; effect of testosterone propionate on, 1314*
- during progesterone therapy of uterine leiomyomas, 1273*
- in 8-year-old girl with ovarian dysgerminoma, 1349*
- lactation, cessation of; effect on hyperthyroidism, 330*
- persistent lactation in acromegaly, 126*
- prolactin; assay of, in breast carcinoma, chronic cystic mastitis and other endocrine disorders, 653†

GYNECOMASTIA

- associated with corticoadrenal tumor, 255*, 791*
- associated with feminizing androblastoma testis, 301*
- enlargement of nipples in male dog with feminizing testicular tumor, 579*

BREAST (cont.)**GYNECOMASTIA (cont.)**

- in paraplegic males 457*

BRITISH AMERICAN EXCHANGE FELLOWSHIPS in cancer research, awarded by the American Cancer Society, 575

BROMINE

- replacing iodine in thyroxine; effect, 1099*, 1107*
- sodium bromide, unable to inhibit iodine-deficient goiter, in rats, 1107*
- tetraethylammonium bromide, in diagnosis of pheochromocytoma, 478

CALCIUM METABOLISM: *see also* Blood, calcium; Bones; Electrolytes; Parathyroids

- calcium balance in Cushing's syndrome with "steroid diabetes," 672†
- calcium retention correlated with potassium loss, following single dose of DOCA, 660†
- hypercalcemia in breast cancer with bone metastases; effect of androgen therapy on, 1314*
- hypercalcemic syndrome associated with androgenic and estrogenic therapy, 1*
- in pseudohypoparathyroidism, 862*, 665†
- recalcification of osteolytic and decalcification of osteoplastic metastases to bone from breast cancer, during androgen therapy, 1314*
- spontaneous hypocalcemia and electrocerebral dysfunction, 398

CANCER: *see also* under various organs involved; and under Tumors

- destruction of metastasis of thyroid cancer, treated with radioiodine, 1122*, 1138*
- incidence of, in endocrine case histories, 682†

CARBOHYDRATE METABOLISM: *see also* Adrenals; Blood, sugar; Diabetes mellitus; Insulin

- citric acid response to oral glucose; abnormalities in diabetes, 400
- dextrose tolerance test: *see* Blood, sugar
- effect of implanted PZ insulin on, in normal and diabetic rabbits, 818*
- following ACTH intravenously, 593*
- glucose and galactose administered simultaneously; metabolism of, in man, 670†
- glucose tolerance test: *see* Blood, sugar
- glycine and total amino acids in various pathologic conditions, 398

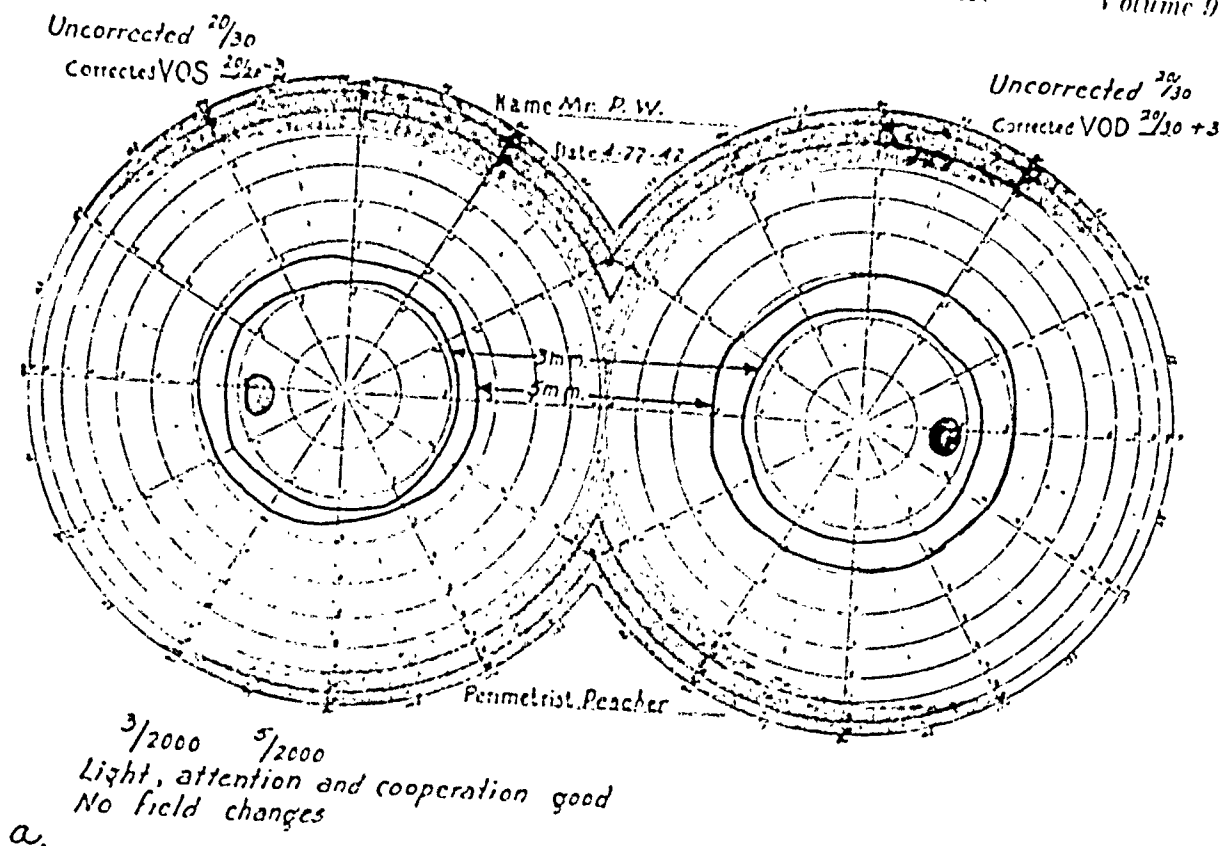


FIG. 1a. Visual fields. Case 7, male aged 20. April 27, 1942. First observation.

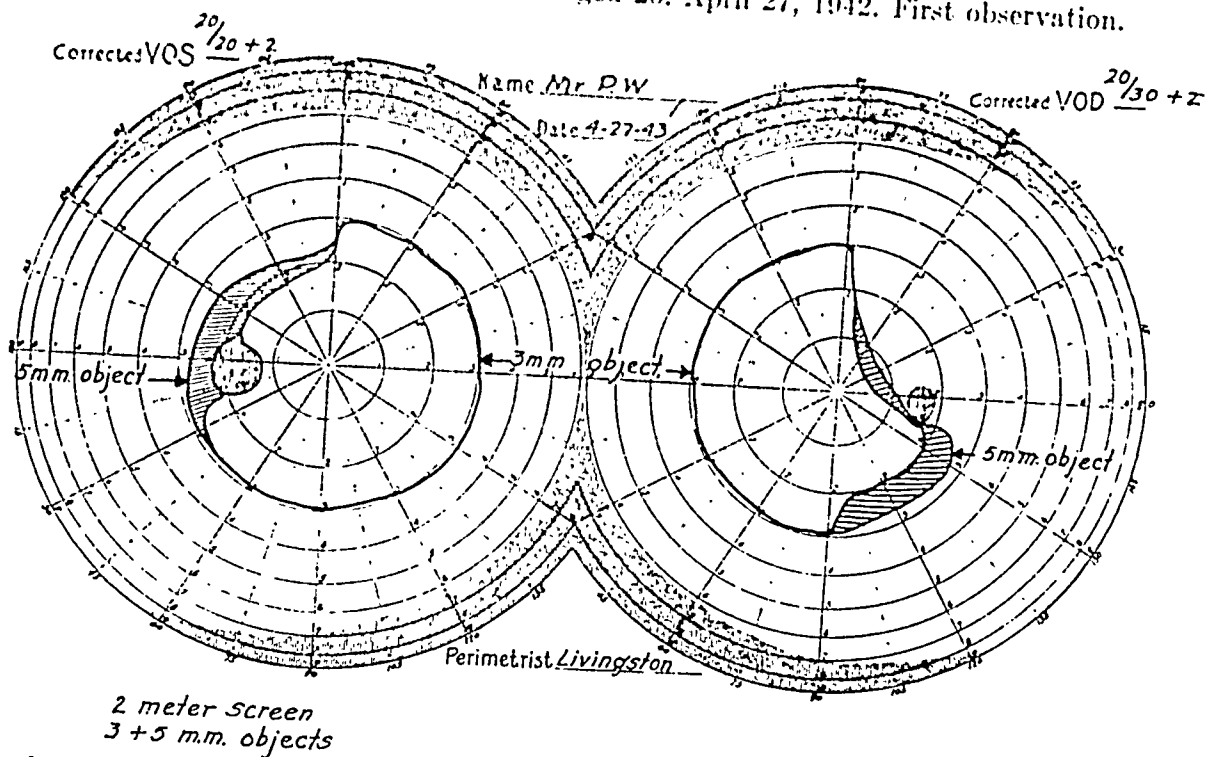


FIG. 1b. Visual fields. Case 7, male aged 20. April 27, 1943. Bitemporal visual field defects which should have indicated further therapy, were not noted by patient.

TABLE 5. CHANGES IN SIZE OF SELLA TURCICA FOLLOWING RADIATION THERAPY

	Measurements in millimeters	Measurements in square millimeters	Period of time between measurements	Number of treatments* between measurements
<i>Decrease in size</i>				
Case 1	16×19 to 14×19	267 to 161	13 yrs.	32
Case 2	28×35 to 21×35	1020 to 601	2 yrs.	13
Case 3	14×17 to 13×15	211 to 184	6 yrs.	42
Case 6	14×15 to 11×13	210 to 168	3 yrs.	24
Case 12	21×22 to 19×22	448 to 388	4 yrs.	12
Case 17	18×19 to 15×19 to 19×20	285 to 252	1½ yrs.	6 (postop.)
Case 18	17×20 to 16×17	360 to 329	16 mos.	12 (postop.)
Case 20	20×11 to 21×10	178 to 161	5 mos.	7 (preop.) 8 (postop.)
<i>Increase in size</i>				
Case 5	17×12 to 19×14		7 yrs.	2
Case 8	20×21 to 21×22 to 23×25	347 to 393	4 yrs.	36
Case 9	15×16 to 15×18 to 14×18	215 to 250	1½ yrs.	24
Case 10	10×15 to 12×15		1 yr.	12
Case 7	rt. 15×23 to lt. 22×23 to rt. 22×25 to lt. 30×34	258 to 426 470 to 793	3 yrs.	18
Case 19	14×20 to 15×20	210 to 254	5 yrs.	18 (preop.) 6 (postop.)

* Each treatment 300r.

an increase in sellar size occurred. In one, this took place in spite of radiation and surgical removal of the tumor, and in another it occurred after adequate roentgen therapy.

A decrease in the size of the sella was noted in 8 instances. In one case (Case 17) the decrease resulted after operation and radiation, but the sella again began to enlarge three years later (*see* section on surgery). The

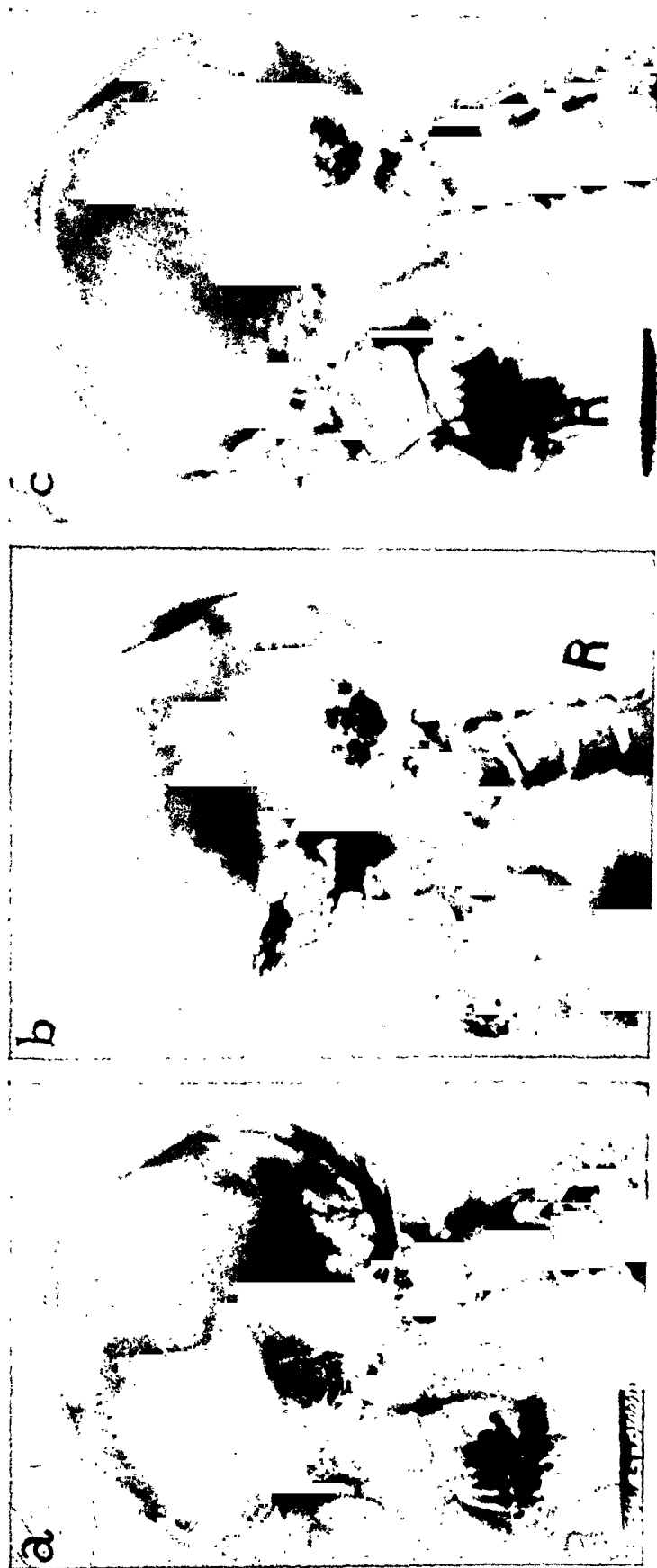


FIG. 2. Roentgenograms of skull. Case 7, male aged 20. *a.* March 19, 1942, first examination; no visual field defects (condition stationary).

b. April 27, 1943, second examination; no change in skull detail or size of sella was observed at that time by routine method; visual field defects had appeared. In retrospect, using the method of measuring the area in square millimeters of lateral contours of both sides as seen stereoscopically, there was further enlargement of the left side posteriorly (see Fig. 3).

c. January 29, 1945. Note further enlargement of sella and frontal sinuses. Marked visual field defect was present.

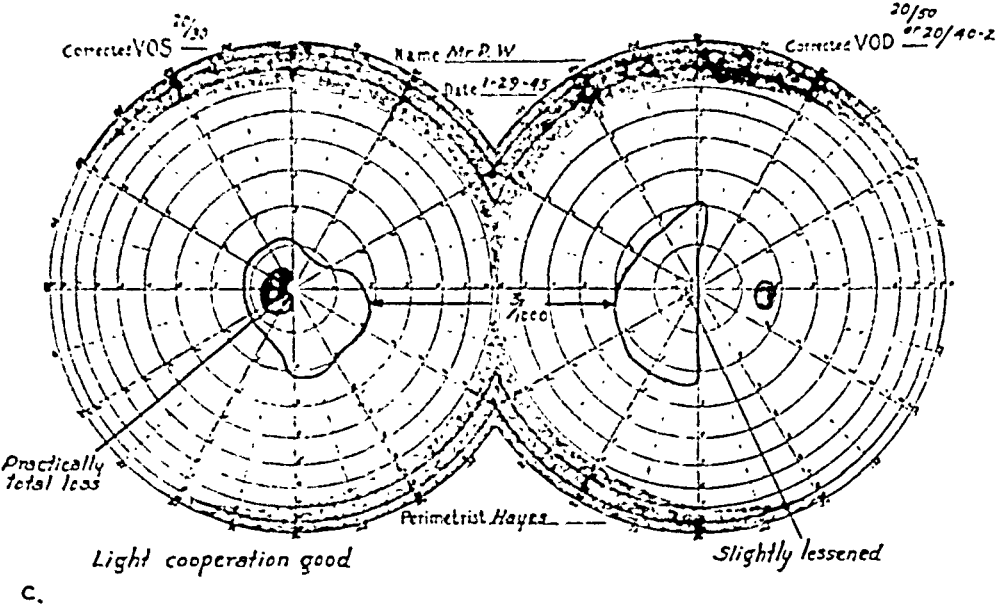


FIG. 1c. Visual fields. Case 7, male aged 20. January 29, 1945. Further decline in visual fields and acuity. Patient noted change in vision. Therapy begun.

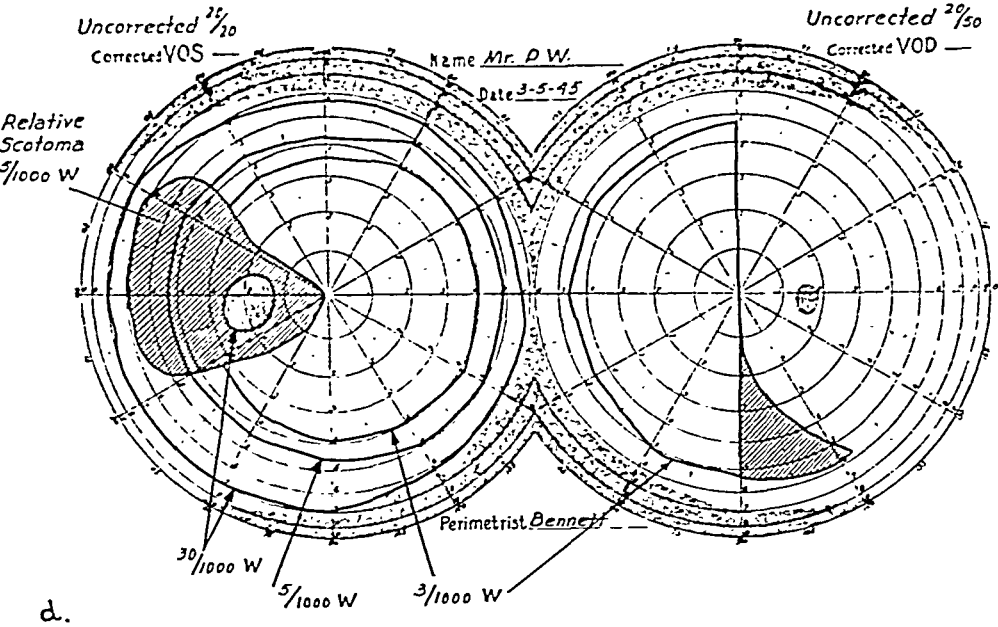


FIG. 1d. Visual fields. Case 7, male aged 20. March 5, 1945. Visual fields showing marked improvement after six roentgen treatments.

the duration. Others, of course, had inadequate radiation therapy, and some had sufficient amounts without results (Case 8, thirty-six treatments).

Thus, it appears that temporary relief can be obtained in some patients with a relatively small number of treatments (Case 12, twelve), whereas others require a much larger dosage. In some instances relief seems unattainable even with adequate therapy and, as occurred in one case, even surgical removal of the tumor did not completely relieve headaches. Analgesics, such as aspirin or empirin compound are rarely of great benefit.

5. Catamenia

Perhaps one of the best indications of success by radiation therapy is the subsequent re-establishment of menstruation. Tumor pressure on pituitary cells producing gonadotropic hormones or pressure upon the pituitary stalk is probably the cause of amenorrhea, rather than excess gonadotropins from an over-active adenoma. It has been shown that this symptom rarely occurs if the sella is within normal limits. Urinary gonadotropins (FSH) may be absent or reduced (8) except in those patients past the time of usual menopause; however, there is no constant pattern. A corollary exists in pituitary gigantism in which sexual development is retarded in spite of excessive growth.

Occasionally menorrhagia may develop, which if excessive, may require drastic procedures (*i.e.*, x-ray or surgery) if medical measures such as large doses of progestin or testosterone are of no avail.

6. Hyperthyroidism, diabetes and persistent lactation

Radiation therapy was given in one case of pituitary adenoma with hyperthyroidism. Although only twelve treatments were given, no improvement was noted. Another patient (Case 4) with a metabolic rate of plus 43 per cent and active diabetes, however, had a decrease in basal metabolism to plus 7 per cent, together with an apparent improvement in glucose tolerance, although the latter may well have been due to more careful diabetic management.

Persistent lactation was unaffected by roentgen therapy in one case (Case 8), but was lessened by stilbestrol in 5 mg. doses parenterally once a week. Larger doses might have been tried.

SURGERY

During the period 1932 to 1945, 5 patients with acromegaly had pituitary operations (Table 3). Two young females (23 and 28 years of age—Cases 17 and 18) had a surgical procedure because only a few acromegalic changes had occurred along with visual field defects, headache and amenor-

most marked decrease which we have seen occurred in a case of pituitary gigantism not included in this series but published elsewhere (7). Following radiation therapy the sella decreased from 23 by 19 mm. to 19 by 13 mm. Recalcification of the clinoids and floor of the sella has been noted in some cases without change in size of the sella. Such alteration probably can be interpreted as relief of pressure from the expanding tumor.

4. Headache

Headache in acromegaly follows no constant pattern. In only a few cases can it be attributed definitely to the expanding tumor, although this point

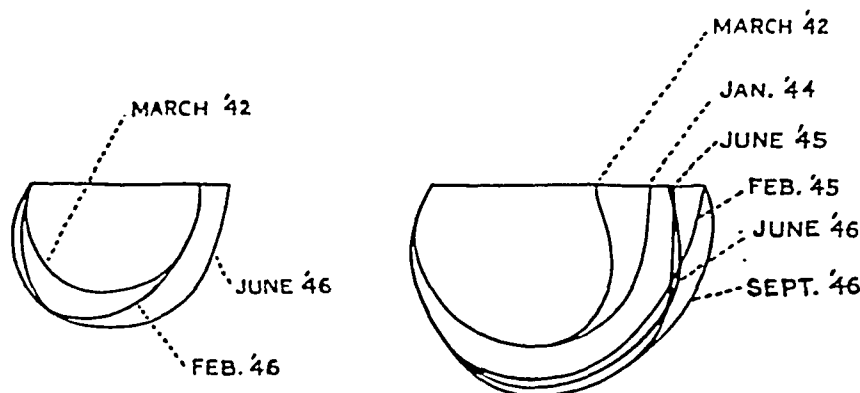


FIG. 3. Lateral contour tracings of sella turcica in acromegaly (Case 7). Note disproportional and progressive enlargement of left side. Minor changes in lateral contour area are not too significant in view of the difficulty in accurately tracing borders of sella from stereoscopic films. Contour lines represent only those in which a change was noted from previous films.

is difficult to settle. Evidence of local pressure is found in some cases at operation when not suspected beforehand. The finding of increased spinal fluid pressure is, of course, an indication for air-injection studies in order to detect any intracranial extension of the tumor. The variability of headache suggests its hormonal origin in some patients. Relief can be expected, at least temporarily, following radiation but one has to consider the possible psychic effect of this treatment in evaluation of such a subjective symptom. Other objective changes are more reliable for determining effectiveness of radiation but such changes are not always forthcoming.

In only one of our cases was headache completely alleviated for a period of two years after the last series of radiation. The greatest number of treatments given to any one patient (Case 3) was forty-two over a period of six years, with only temporary cure of headache following each series. Several patients had marked relief, but no follow-up report has been obtained as to

TREATMENT OF BONY CHANGES

Osteomas may become large enough to produce symptoms and require removal. One of our patients (Case 23) had a large osteoma of the orbit and this was extirpated surgically.

Hypertrophic arthritis produced symptoms in 5 patients. Treatment in these cases is either on a symptomatic or orthopedic basis. Patients who displayed marked arthritic changes had had the disease for at least eight years or more; earlier and more persistent roentgen therapy might have retarded the development of these changes.

TREATMENT WITH ESTROGENS, TESTOSTERONE AND GONADOTROPINS

Estrogenic preparations have been given to some of our patients for various purposes. Hot flashes in women past the menopause may continue in spite of treatment for acromegaly, and estrogens are useful. In 2 cases (Cases 6 and 19) weekly intramuscular injections of 5 mg. of stilbestrol administered for several months were not successful in ameliorating headache. The same medication used for persistent lactation (Case 8) appeared to decrease the flow and to relieve headaches. A male (Case 7) received large doses of estrone sulfate and stilbestrol (up to 15 mg. daily). This produced slight breast development, some loss of body hair, and loss of the visual improvement which followed radiation therapy a few months before (Fig. 2). Further enlargement of the sella also took place so that surgical removal has since been done (1946). Cognizant of the fact that Zondek (9) produced marked enlargement of the hypophyses in rats with large doses of estradiol benzoate (given subcutaneously and percutaneously), it occurred to us that estrogen therapy might have increased the size of the tumor in this case. While estrogen therapy may inhibit the growth factor and decrease blood phosphorus as it did in one instance (Case 10, Table 1) the possibility of simultaneous enlargement of the tumor with further visual change had to be considered. The beneficial results of estrogen therapy in acromegaly have been reported by several writers (10-13).

A review of our cases in which further enlargement of the sella took place after therapy, either surgical or x-ray, shows that 6 of the 7 patients received estrogens; whereas only 1 of the 7 who had a permanent decrease in size received estrogen therapy. Whether or not estrogens are contraindicated is difficult to say, since they were administered usually to those patients in whom there might be reason to believe progression was already under way. Further observations are necessary.

We have not used testosterone in any of the male patients in this series, chiefly because none of these patients who have been under our continual

rhea. Both operations were followed by six x-ray treatments of 300 r each. Headaches were relieved (twenty-four months), but amenorrhea has persisted. No further acromegalic changes have resulted; in fact, there was excellent regression of tissue changes in one and moderate alteration in the other. The latter patient gained excessive weight whereas in the former, hot flashes have persisted (age 23). This patient had persistent anemia and complained of lassitude for thirty-six months after operation, but at the time of the last visit both had improved. An enlarging sella, however, indicated recurrence, for which x-ray therapy has been instituted.

In another patient, age 33 (Case 19), who received inadequate radiation therapy largely because of the inaccessibility of a place where this could be given, and because of fairly rapid enlargement of the sella with increasing headache, an operation was performed. Considerable regression of tissue changes followed but complete relief of headache was only temporary, there being some mild head discomfort when she was last seen. Menstruation returned fairly regularly in this case, as it had previously after a course of radiation therapy. Operation could be considered successful to date, so far as prevention of further acromegalic changes and restoration of normal catamenia are concerned, but only partly so with regard to headache. Only questionable visual field changes were present before operation.

A fourth patient (Case 15) had had forty x-ray treatments in two years, and then had several operations, one in 1932 and another in 1937. She died elsewhere in 1940 following an exploration attempted because she had become semicomatose. Improvement in this case followed each of the first two operations but lasted only a few years. At the first operation the tumor had extended into the floor of the third ventricle, making subtotal extirpation hazardous. Had operation been performed earlier in the course of her disease, or if more vigorous radiation had been given, this patient might have been saved.

The only male (Table 3, Case 16) subjected to operation up to 1945 was a patient aged 30 who had received no benefit from radiation therapy elsewhere; he was operated upon because of headache. Exploration revealed much edema but only a small pituitary adenoma (sella enlarged) which was not removed. Relief of headache was only temporary. He died of leukemia nine months later; his blood counts at the time of operation were normal. Acromegaly had been present only two years.

Of 4 cases in which the pituitary tumor was removed, the pathologic report was eosinophilic adenoma in 2 and mixed type in 1. The material removed in the fourth case was unsatisfactory for microscopic diagnosis, presumably because of degeneration of tumor cells.

TREATMENT OF GOITER AND HYPERTHYROIDISM

Only 3 cases in this series showed active hyperthyroidism. Two patients were operated upon with the usual preparation and an excellent result obtained. This is not a universal experience (15). A third patient (Case 12) was a man of 40 with a very large goiter, and an initial metabolic rate of plus 70 per cent. He derived no benefit from irradiation of the pituitary or from iodine. He was given thiouracil and his condition improved. The metabolic rate dropped from plus 57 per cent to plus 11 per cent. Subtotal thyroidectomy was then performed without any postoperative reaction. The patient remained well until recently, thirty-six months after operation, when there appeared to be a recurrence of hyperthyroidism (Table 2, Case 12). The metabolic rate was plus 25 per cent. Treatment with propylthiouracil has been started. There was no obvious increase in other aspects of acromegaly or any mental or neurologic change. In a recent case not included in this series, a nodular goiter was present which caused sufficient compression of the trachea to produce stridor. The goiter was removed surgically without antithyroid drug preparation, as there was no evidence of hyperthyroidism.

MENTAL AND NEUROLOGIC CHANGES

Mental changes are probably more frequent than have been noted. It seems to us that personality changes may occur in a goodly number but may be entirely unsuspected because of their gradual development. As mentioned before, an acute psychosis followed radiation therapy in one case. Paranoia was present in another, but radiation treatment was not attempted. Our impressions as to personality deviations following radiation therapy are vague and no reliable conclusions can be drawn.

Convulsive seizures developed in 1 case but did not recur until one year after radiation therapy. No further contact has been made with this patient.

CAUSES OF DEATH

Of the 29 patients who form the subject of this report, 7 are known to be dead. The causes of death in the 7 cases were: 1) local regrowth and extension of the tumor; 2) diabetic coma; 3) pneumonia during psychotic state; 4) subacute bacterial endocarditis; 5) coronary infarction; 6) leukemia, and 7) meningitis, two months after satisfactory operation.

IMPORTANCE OF REGULAR EXAMINATIONS

Periodic examinations should be advised for all patients with acromegaly. This should be impressed upon them because of the possibility of a rapid

observation has developed significant hypopituitarism or secondary hypogonadism. In one female (Case 3) testosterone pellets (75 mg.) were implanted as a trial procedure for relief of headache. The patient reported sufficient improvement to desire a second implantation. From our experience in the treatment of patients with chromophobe tumors without acromegaly, beneficial results may be expected from testosterone therapy should hypofunction arise in the course of acromegaly (12, 13). Weekly doses of 50 to 100 mg. of testosterone propionate parenterally should suffice. Implantation of testosterone pellets in a dose of 150 to 300 mg. is effective and at times preferable to the parenteral method. Methyltestosterone in daily oral or sublingual doses of 20 to 50 mg. may be helpful. If evidence of adrenal insufficiency appears, desoxycorticosterone or adrenal cortical extract may be used.

Pituitary gonadotropic or chorionic preparations have not been used in any of our patients with acromegaly, although definite results were obtained in a case of pituitary gigantism (7). They might induce ovulation in cases of amenorrhea occurring from suppression of gonadotropic principles. Large doses of these preparations, either singly or combined, would have to be given. Such priming has doubtful value, although Lerman (14) believed good results were obtained with pregnant mare urine in a case of severe pituitary myxedema. In one of our cases with severe hypopituitarism, doses of from 25 to 50 units¹ per day for a period of a month failed to produce any change.

THERAPY FOR MISCELLANEOUS SIGNS AND SYMPTOMS

For lassitude, which is a frequent complaint, particularly when pituitary activity is ebbing, nothing has greater effect in our experience than benzedrine sulfate (in doses of 10 to 30 mg. per day). Desiccated thyroid, even in cases of marked hypopituitarism, may not be well tolerated and is not of great value. Anemia which occurs in the hypopituitary state seems to be unaffected by liver or iron, but may be improved slightly to moderately with testosterone. A marked improvement in anemia may indicate a recurrence of tumor growth, or reestablishment of normal pituitary function (Case 17).

Severe hypertension may occasionally be a problem and the usual palliative measures should be tried. In none of the cases in this report was there hypertension of severe degree. In a recent case showing a high diastolic pressure little benefit was derived in this regard with roentgen therapy.

¹ Courtesy of The Upjohn Company.

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enlargement of the sella from the expanding tumor or sudden hemorrhage. This is not always preceded by an increase in other signs and symptoms.

Some individuals with this disorder have so few complaints and the immediate results of roentgen therapy are so slight that it is occasionally difficult to persuade them that further treatment is necessary. Goldberg and Lisser (16) particularly have stressed the importance of periodic examination of acromegalics. We wish to re-emphasize this point of view, believing that best results of treatment depend largely upon it.

CONCLUSIONS

Earlier recognition of acromegaly is paramount if prevention of the disfiguring aspects of this disease is to be expected.

The finding of an elevated blood phosphorus level may prove to be a valuable indication of active acromegaly.

Radiation therapy should be given before operation, at least for a trial period of three to six months. In young females especially, operation should be advised if the disease is not controlled after six months of adequate radiation therapy.

More intensive radiation therapy should be given than has been employed in this series of cases.

Patients with acromegaly should be seen at least once or twice a year even if asymptomatic; visual fields and roentgenologic examinations should be done.

Surgical treatment is indicated whenever irradiation therapy does not prevent progression of the disease or further enlargement of the sella.

The possibility of increasing the size of the pituitary adenoma by estrogen therapy must be considered.

Addendum

Addendum: Since this article was submitted for publication, Kinsell *et al.* have noted that the simultaneous use of testosterone and estrogen will prevent the feminizing changes which were prominent in Case 10 of this series. (Kinsell, L. W., Michaels, G. D., Li, C. H. and Larsen, W. E.: Studies in growth. I. Interrelationship between pituitary growth factor and growth-promoting androgens in acromegaly and gigantism. II. Quantitative evaluation of bone and soft tissue growth in acromegaly and gigantism, *J. Clin. Endocrinol.* 8: 1013-1036 (Dec.) 1948.)

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graphic technique (1). For this purpose, the histologic sections were coated with fluid emulsion and allowed to dry in the dark. The emulsion was thus bombarded by the radiations from the radioactive iodine present in the section. On development, dark silver granules overlay the sections at the sites of radioiodine deposition. This radioiodine in the sections was organically bound, since the tissues were fixed, mounted in paraffin and stained, and therefore the iodine present as iodide was extracted. The radioiodide detected by this technique was presumably bound as thyroglobulin.

The animals given a daily iodine supplement showed the presence of radioiodine in the follicular epithelium at one hour after injection. This was indicated by the circular pattern of the photographic reactions which corresponded to the arrangement of the cells (Fig. 1). Examination of

TABLE 1. FIXATION OF I^{131} IN THE THYROID OF RATS GIVEN VARIOUS DOSES OF IODINE

Group	No. of animals	Per cent of injected dose	
		1 hr. after injection	24 hrs. after injection
Control animals	4	10.5	53.5
(Remington diet)		range (21.2-3.05)	range (58.0-43.5)
Animals receiving drinking water	4	1.7	8.3
containing 2 γ of iodide per cc.		range (1.0-2.4)	range (7.5-8.9)

lightly exposed sections under the high power of the microscope revealed that the radioactivity was located either at the apex of the cells or in the peripheral portions of the colloid (Fig. 2). It may be recalled here that histologists have long noted that the apex of the thyroid cell is often the site of appearance of small colloid globules staining like the colloid itself. Whereas in most of the follicles the radioactivity was located in or near the apex of the cell, it was possible to find some follicles showing a reaction throughout the colloid, especially in the central areas of the thyroid (Fig. 1).

In contrast, twenty-four hours after injection of radioiodine, similar animals revealed a uniform distribution of the radioiodine throughout the colloid of their follicles (Fig. 3). Under the high power of the microscope it could be confirmed that the black granules of the photographic reaction were most abundant in the colloid (Fig. 4).

Comparison of the results obtained at one and at twenty-four hours

THE MECHANISM OF THE SECRETION OF THYROID HORMONE*

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INTRODUCTION

THE coupling of iodine with a protein is an essential part of the activity of the thyroid gland. How this reaction leads to the formation of the hormone is nevertheless obscure, for the nature of the hormone is still unknown.

In this paper the autographic technique was used to visualize the formation of an iodinated protein in the apex of the thyroid cell and its passage into the colloid. By means of isotope dilution it was shown that this protein releases within the thyroid the amino acid thyroxine, which then enters the blood. These results point to thyroxine as being the form in which the hormone is released by the gland and circulates in the body.

Method: The various phases of this work were carried out with rats receiving Remington's low-iodine diet, which supplies about 2 micrograms of iodine daily per 100 Gm. rat. Additional animals received a supplement of 20 micrograms of iodine per day, either by injection or in the drinking water. After a minimum of six weeks on the diet, the animals were injected with carrier-free radioiodine. Two types of investigations were carried out: 1) a histologic localization of the radioiodine entering the thyroid, and 2) an attempt to identify some iodinated compounds in thyroid and blood by means of isotope dilution.

VISUALIZATION OF THE IODINE ENTRY INTO THE THYROID

The animals on the low iodine diet showed active thyroids, since histologic examination of the follicles revealed high columnar cells and narrow lumina, and the radioiodine uptake was about half the injected dose twenty-four hours after injection. The animals given a daily iodine supplement had a less active gland with lower epithelium and wider follicular lumina, and a decreased radioiodine uptake (Table 1).

The histologic location of the radioiodine was examined in the thyroid sections of these various groups of animals with the help of the auto-

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after injection in the animals receiving the high iodine diet made it possible to follow step by step the fate of the iodine taken up by the thyroid gland. The transformation of the iodide entering the thyroid into an organic compound—presumably thyroglobulin—was visualized in the apex of the cell. From this location the organic iodine had invaded the whole colloid twenty-four hours later (Fig. 4). It must be kept in mind that the radioiodide was carrier-free and therefore indicated the behavior of the small amounts of iodine always present in the blood. Therefore, these results revealed iodine transformations which are taking place in the thyroid gland under physiological conditions.

In the animals on a low-iodine uptake, the radioactive iodine was present in the colloid at one and twenty-four hours after injection, with no reaction being apparent in the cells. Therefore, the radioiodine must have crossed the cellular barrier and become transformed into thyroglobulin within a very short time.

A reaction was present in all thyroid follicles of all animals. Therefore, all follicles must be simultaneously active in building up organic iodine from the circulating iodide and depositing it in the colloid. Some follicles in the rat thyroid (the central ones) work at a faster rate than others (the peripheral ones). Presumably, each follicle has its own rate of formation and excretion of organic compounds, *i.e.*, its own "steady state"; but all of them are continuously contributing organic iodine to their colloid.

RELEASE OF IODIZED COMPOUNDS FROM THE THYROID

While numerous theories have attempted to explain the release of iodized compounds from the thyroid gland, De Robertis' hypothesis (2) has so far supplied the most satisfactory explanation. This author demonstrated the presence of a proteolytic enzyme system in the colloid of thyroid follicles. This enzyme was presumed to transform thyroglobulin into smaller molecules reaching the blood stream by diffusion through the thyroid cells. The recent finding of free thyroxine in the blood (3, 4) suggested the possibility that the proteolysis of thyroglobulin in the thyroid yielded thyroxine.

It is widely held that thyroxine is not present as such in the thyroid gland (5). However, it was possible that amounts of free thyroxine and free diiodotyrosine too small for chemical identification could be recovered by the method of isotope dilution. This method consists of identifying minute amounts of a radioactive substance with the help of large amounts of the same substance in an inactive form. Thus, a large amount of inactive thyroxine was added to the thyroid gland of a radioiodine treated animal and purified by successive recrystallizations. This inactive thyroxine should mix with the free radiothyroxine if present, and carry it

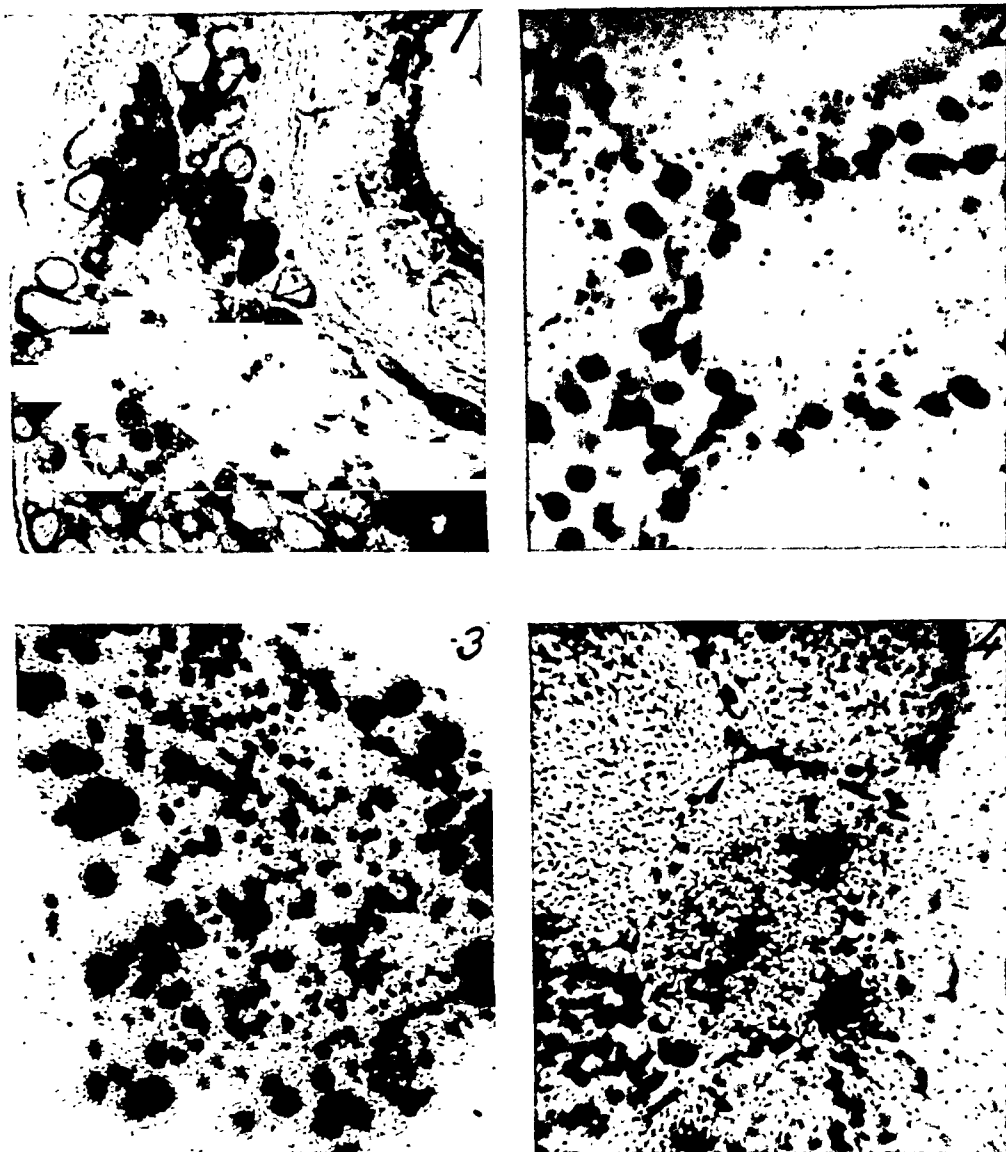


PLATE 1. These photomicrographs represent autographs of the thyroids of animals on a high-iodine diet (22 micrograms daily) given radioactive iodine. The sections have been coated with photographic emulsion which on development showed the accumulation of black granules over the site of the radioactive iodine.

FIG. 1. (about $\times 75$) shows the thyroid under medium power at 1 hour after I^{131} administration. Note that the radioactive iodine is present in the form of rings corresponding to the outlines of the follicles. Under higher magnification (Fig. 2., $\times 600$) the iodine can be shown in the apical portions of the cells and the periphery of the colloid. Figs. 3 (about $\times 75$) and 4 ($\times 450$) are corresponding autographs of the thyroid removed 24 hours after I^{131} administration. Note that now iodine is located uniformly throughout the colloid and in higher concentration (areas are blacker) than in the cells.

TABLE 2. PROTOCOL OF A TYPICAL EXPERIMENT

A female albino rat on a diet containing 0.2 microgram of iodine per gram was injected subcutaneously with a carrier-free dose of radioactive iodide. After 24 hours the animal was killed and the thyroid and plasma were treated as described in the text.

	Radioiodine content (counts per minute)	Per cent of in- jected dose	Per cent of I^{131} present in thyroid proteins
Residual thyroid proteins after butanol ex- traction	3,290,000	38.00	100.0
Thyroxine in butanol extract of thyroid sus- pensions (determined from isotope dilution)	35,500	0.4	1.1
Thyroxine in butanol extract of total plasma (determined from isotope dilution)	259,000	2.8	7.8

which De Robertis has identified a proteolytic enzyme system. The presence of thyroxine in the blood indicates that this substance is capable of diffusing from the follicle into the extracellular fluids and the circulation.

A more detailed comparison of the values for free thyroxine in thyroid tissue and in plasma (Table 3) indicated that the ratio of the concentration in the thyroid to that in the plasma was similar in 4 out of 5 experiments. This ratio indicated that the free thyroxine was on the average, 23 times more concentrated in thyroid tissue than in plasma. Thus, there exists a

TABLE 3. AMOUNTS OF LABELED FREE THYROXINE IN THYROID AND PLASMA AT 17 HOURS AFTER INJECTION OF RADIOIODIDE

Experiment number	Per cent of injected radioactivity present as free thyroxine		Ratio of concentration* of free thyroxine in thyroid over concen- tration of free thyroxine in plasma
	In thyroid	In total plasma	
1	0.08	0.54	23
2	0.20	3.44	7
3	0.38	1.42	29
4	0.13	0.47	27
5	0.08	0.34	28
Average	0.17	1.24	23

* The concentrations were calculated as the per cent of injected radioactivity present as free thyroxine per milligram of thyroid tissue or per milligram of plasma.

when precipitating or crystallizing. The absence of radioactivity in the precipitate would indicate that no free thyroxine is present in the gland. Conversely, if the precipitate were to carry a constant concentration of radioactivity, the presence of free radiothyroxine in the gland would be indicated. The constancy of the concentration is shown by the constancy of the radioactive count per microgram of substance (or specific activity) on repeated recrystallizations.

Adult rats on a low-iodine diet were sacrificed at 17 ± 2 hours after administration of a tracer dose of radioiodine. Their thyroid glands were quickly ground in the cold with 2 cc. of cold saline and shaken three times with a double volume of butyl alcohol. The butyl extract was then washed with 3 portions of 4 normal NaOH containing 5 per cent Na_2CO_3 . Fifty

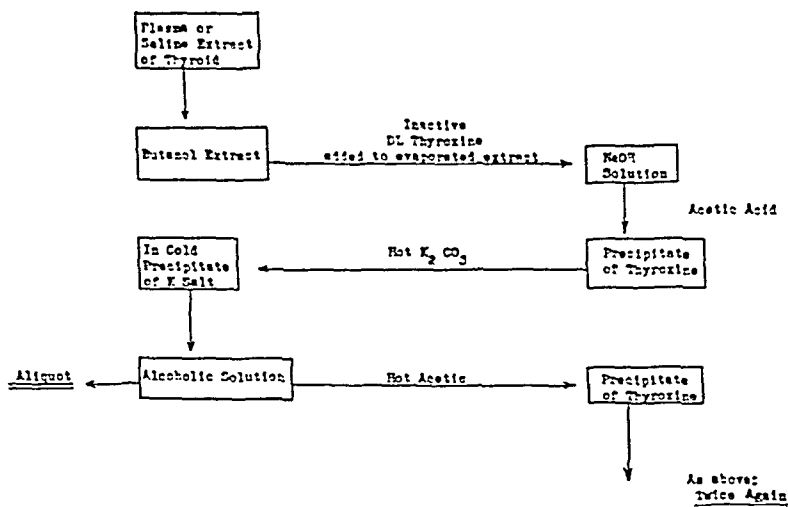


FIG. 5. Isotopic dilution of thyroxine.

milligrams of thyroxine was added to the butyl extract. Thyroxine was purified through precipitation from hot tenth-normal K_2CO_3 by cooling and from alcoholic and alkaline solutions by acetic acid (Fig. 5). This sequence was repeated three times. A fairly constant specific activity was obtained after the first precipitation series. Similar results were obtained with blood plasma, in which no initial drop in specific activity occurred (Table 2, Fig. 6). It was concluded that free radioactive thyroxine was present in thyroid tissue and plasma. The alternate possibility was that a compound very closely related to thyroxine may have behaved in a similar manner in the course of the isotope dilution.

These results are best explained by assuming that thyroglobulin is hydrolyzed with the liberation of free thyroxine in thyroid tissue. This hydrolysis presumably takes place in the colloid of the thyroid follicle, in

It may be noted here that isotope dilutions, carried out with diiodotyrosine instead of thyroxine suggested the presence of free diiodotyrosine in thyroid tissue and blood plasma. However, the possibility of exchange taking place in the case of this substance has not been eliminated. Therefore, a final decision cannot be made in regard to the presence of free diiodotyrosine in the gland.

It was possible that the relatively large amounts of radioactivity used in these experiments (on the average, 80 microcuries) might have produced some breakdown of thyroxine from thyroglobulin. This possibility was eliminated by demonstrating the presence of the free compound in thyroid tissue and blood of animals treated with doses of 0.5 microcurie.

While all these investigations were carried out in animals receiving a low-iodine diet, it was possible that free thyroxine was not present under more normal conditions, that is to say, with a higher iodine level in the diet. This possibility was investigated by repeating the isotope dilution in a purina-fed rat. Under these conditions the presence of free thyroxine in the thyroid and blood was also demonstrated.

In summary then, the following points appear. An iodinated compound may be extracted by *n*-butanol from unhydrolyzed thyroid tissue and plasma. On isotope dilution with thyroxine, this substance was found to behave like thyroxine through nine successive recrystallizations (*i.e.* from alkaline and alcoholic solution by means of acid, and from hot potassium carbonate solution, by cooling). While this behavior might be due to a compound closely related to thyroxine, it seems more likely that this butanol-soluble material is *l*-thyroxine itself. Since it was found that the concentration of this compound in the thyroid was considerably greater than that in the plasma, it would seem that there is a passage of thyroxine from the gland into the blood. Further, since the greater part of the plasma radioactivity was found in the butanol extract, it seems likely that this substance represents the hormone of the thyroid gland and in fact that the hormone is *l*-thyroxine.

The possibility that thyroxine circulating in the plasma is bound to a protein cannot be excluded, but in view of the gentle extraction methods used, this protein-hormone combination must be very loose indeed.

CONCLUSION

The secretory mechanism of the thyroid gland includes the following steps:

1. The iodide taken up from the blood by the thyroid cell is incorporated into organically bound iodine (thyroglobulin) in the apex of the cell. It is then deposited as such in the colloid of the thyroid follicle.

gradient sufficient to induce a diffusion of the material from the gland into the circulation.

Several possible causes of error had to be considered. First, there might be an exchange of iodine atoms between the radioactive iodide present in small amounts in the gland, and the large amounts of inactive thyroxine added for the isotope dilution. That this was not the case is shown in Fig. 6, in which the Roman numerals indicate the following: I is the specific

SPECIFIC ACTIVITY
OF THYROXINE

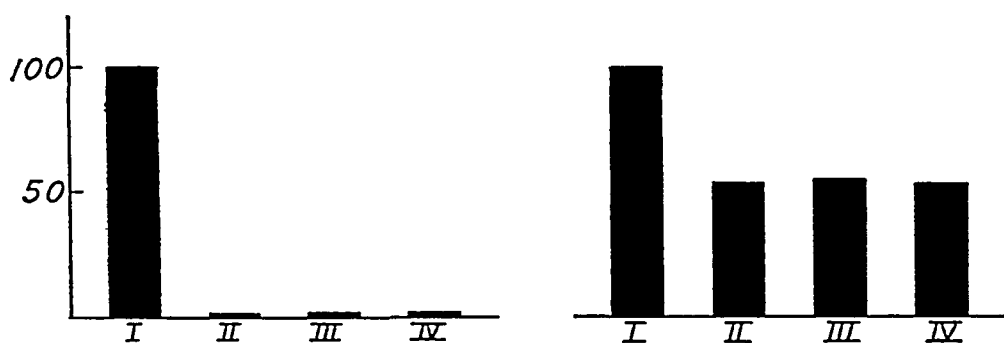


FIG. 6. This chart is a representation of the findings in the isotope dilution of the free thyroxine in the thyroid. The radioactivity per microgram of substance (*i.e.* the specific activity) is represented for the butanol or alkaline extract (I) and for subsequent recrystallizations (II, III, IV). The diagrams on the right were the values obtained in the thyroids of animals which had been injected with I^{131} . The figures on the left indicated specific activities found in control experiments, in which the thyroids of untreated rats were ground with saline containing radioactive iodine.

activity found in the original butanol extract. II, III, IV represent the specific activities on successive series of recrystallizations. The right hand graph represents the specific activities obtained in the thyroids of animals injected with radioiodine. The graphs on the left side (*i.e.* the control experiments) indicate the specific activities obtained when thyroids from untreated animals are extracted with saline containing radioactive sodium iodide. These latter dilutions showed a drop of the specific activity to less than 0.1 per cent. Therefore, there is only a very small amount of exchange, if any, in our experimental conditions; and the previous results showing the presence of free thyroxine in thyroid and plasma may not be attributed to exchange phenomena.

WIDTH OF ADRENAL CORTEX IN LYMPHATIC LEUKEMIA, LYMPHOSARCOMA AND HYPERTHYROIDISM

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THE recent work of Dougherty and White (1), indicating that the adrenal cortex has an important regulatory effect on lymphoid tissue, the demonstration (2, 3, 4) that cortical hormones may influence the course of experimental leukemia and lymphosarcoma, and the finding of a lowered excretion of 17-ketosteroids in lymphatic leukemia (5), raise questions concerning a possible role of the adrenals in human neoplastic disease involving lymphoid tissues.

The well-known hyperplasia of thymus and lymphoid tissue in hyperthyroidism, emphasized by Marine *et al.* (6), Warthin (7) and Friedgood (8, 9), and the suspicion on other grounds that the adrenals may be implicated in hyperthyroidism (10, 11, 12) suggest a possible interrelation of the thyroid and adrenals.

With these facts in mind, measurements of the width of the adrenal cortex were made in a series of autopsies from the files of the New England Deaconess Hospital. This was done on the assumption that the width of the adrenal cortex is correlated with its function, as indeed it seems to be in Cushing's syndrome, some cases of virilism, and in Addison's disease with so-called primary atrophy of the adrenal cortex (13).

MATERIALS AND METHODS

The material consisted of cross sections of adrenal glands taken at autopsy. They had all been fixed in Zenker's fluid with acetic acid, embedded in paraffin and stained with eosin-methylene blue, also usually with Mallory's phosphotungstic acid hematoxylin. It seemed, therefore, that the degree of shrinkage due to processing of the tissue should be uniform for the entire series. Measurements were made by means of a micrometer eyepiece, five different fields being measured on each slide and the results averaged. Cases in which the gland was distorted by postmortem changes or metastatic tumor were rejected. It was not found possible to make a clear distinction between the various zones of the cortex. No attempt was made to deduce the lipoid content from the vacuolization of the cells.

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2. The presence of free thyroxine has been demonstrated in thyroid tissue. This result indicates that the thyroglobulin is hydrolyzed to yield thyroxine, presumably by the proteolytic enzymes contained in the colloid.

3. The presence of free thyroxine has been demonstrated in blood plasma. The possibility that this substance originates from the free thyroxine fraction of the thyroid gland is supported by the presence of a gradient in concentration between the thyroid and blood.

4. It is proposed that *l*-thyroxine is in fact the form in which the thyroid hormone is released by the thyroid and circulates in the body.

Acknowledgment

This work was supported by a grant from the National Research Council of Canada.

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ference between the means is 0.12, less than twice the standard error, which is again 0.08, indicating no significant difference.

When, however, the controls are compared with the hyperthyroid group, the difference between the means is 0.21, which is 4.2 times the standard error of 0.05, indicating in all probability a highly significant difference.

DISCUSSION

If it be granted that the adrenal cortices in this relatively small group of cases of hyperthyroidism were significantly narrower than those in the control group, as they seemed to be, the reason for the difference remains to be sought.²

It may be objected that thyrotoxicosis and especially "thyroid storm" is a severe strain on the organism and might be expected to affect the adrenal glands. Indeed, thyrotoxicosis has been compared (14) to the "alarm reaction" of Selye (15). However, in the latter condition, no matter what the alarming stimulus, the adrenal cortex is uniformly enlarged, not narrowed (15). Furthermore, the majority of patients in the control group in this series were also exposed to considerable stress, being for the most part severely ill and frequently subjected to operative trauma shortly before death. It is true, however, that in most of the controls the termination of the illness was not as sudden or cataclysmic as in the hyperthyroid cases (12 of the 15 were operated on, and more than half died within two days after operation).

The state of nutrition of the patient might also be regarded as influencing the width of the adrenal cortex. Nine of the 15 hyperthyroid patients were described as fairly well nourished, two as "extremely emaciated" and the rest as "thin" or "poorly nourished." (As a matter of fact, one might expect an enlargement of the adrenal cortex under conditions of malnutrition since Selye has described such enlargement in the experimental animal during fasting).

It might also be objected that the control group, representing as it does a heterogeneous collection of diseases, might be overweighted with conditions thought by some to be associated with enlargement of the adrenal cortex, as for instance hypertension, cancer or infectious disease. It was found that 10 of the 50 control cases had a definite history of hypertension; the mean width of the cortex in these 10 cases was 1.18 mm. and when they were eliminated, the mean for the control group was still 1.16 mm. Eighteen of the 50 controls had cancer; the mean cortical width in these cases was 1.07 mm. and their elimination led to an increase of the mean

² It is recognized, of course, that the adrenals of the control group are not in any sense to be regarded as "normal."

The cases consisted of, first a *control group* of 50 cases, which were routine autopsies taken consecutively except for an occasional case discarded for reasons just cited. Half of these control cases were taken from the years 1933-1937, when about half of the cases of hyperthyroidism also occurred (this to control possible minor variations in histologic technique). Secondly, a group of 14 cases of *lymphatic leukemia* was studied; thirdly 13 cases of *lymphosarcoma* and *lymphoblastoma*¹; and finally, 15 cases of *hyperthyroidism*. The latter were mostly postoperative deaths, more than half occurring within two days after either ligation or hemithyroidectomy and showing, in most instances, the clinical signs of "thyroid storm," all had severe Graves' disease, with metabolic rates averaging plus 50 per cent before operation. (In about half the cases the basal metabolic rate had been recorded within five days of operation; in other cases the high rate had been obtained two or three weeks before operation).

The results are summarized in the following table:

TABLE I

Group	No. of cases	Mean width of cortex in mm.	Standard deviation
Control	50	1.16	0.27
Lymphatic leukemia	14	1.22	0.25
Lymphosarcoma	13	1.28	0.23
Hyperthyroidism	15	0.95	0.13

It is apparent from the table that no striking difference occurred among the first three groups, either in the means or in the degree of scatter around the mean, as indicated by the standard deviation. In the last group, however, the mean appears to be distinctly lower than the others, and the standard deviation indicates that the values are closely grouped about the mean (9 of the 15 lay between 0.8 and 1.0 mm., whereas only 9 of the 50 control cases fell in this range; also, only 1 of the hyperthyroid cases had an adrenal cortex larger than 1.16 mm., the mean for the control group).

When the control group is compared with the lymphatic leukemia group, the difference between the means is 0.06 and the standard error of the difference is 0.08, indicating no significant difference.

When the control is compared with the lymphosarcoma group, the dif-

¹ The cases in this group represent malignant neoplastic disease of lymphoid tissue, exclusive of Hodgkin's disease and lymphatic leukemia.

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for the remaining 32 cases to 1.21 mm. In 14 of the 50 controls a bacterial infection appeared to be the principal cause of death; in these 14 the mean cortical width was 1.23 mm., and when they were eliminated the mean for the remaining 36 was 1.14 mm. It appears probable, therefore, that the control group is not overweighted with any particular disease tending to produce enlargement of the adrenal cortex.

The literature on the size of the adrenals in hyperthyroidism, as summarized by Wegelin (16) and by Holst (17), is conflicting, but a reduction in size is described by some authors.

If such a reduction in size (or failure to enlarge) does occur, and if it indicates hypofunction, one may speculate as to whether or not it may be related to the hyperplasia of thymus and lymphoid tissue, mentioned above. Experimental evidence on this point has been difficult to secure, the results suggesting that the thyroid may play a role subordinate to the adrenals in respect to lymphoid tissue, and that there may be a reciprocal relationship between the two, such that hyperplasia of lymphoid tissue might be associated with either hypoplasia of the adrenal cortex or hyperplasia of the thyroid, or both (18, 19, 20). A reciprocal relation between thyroid and adrenals is suggested by the work of the Hoffmanns (12). Other investigators, however, have reported decrease in size of the adrenal cortex following thyroidectomy or treatment with thiouracil, and increase in size after feeding thyroid extract (21, 22).

If adrenal hypoplasia is characteristic of "thyroid storm," one may also wonder whether this hypoplasia indicates a failure of adaptation of the organism in the sense of Selye (15). Clinical evidence concerning adrenal hypofunction in thyrotoxicosis is apparently inconclusive (11, 23).

SUMMARY

The width of the adrenal cortex was measured in autopsy sections in cases grouped as follows: controls (a series of miscellaneous causes of death), lymphatic leukemia, lymphosarcoma, and hyperthyroidism. No significant differences were found among the first three groups. In the cases of hyperthyroidism, however, the adrenal cortices appeared to be significantly narrower than those of the control group. Possible reasons for this difference are discussed.

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hair, seemingly normal libido but potentia marred by inability to ejaculate, and psychic trauma resulting therefrom. Objective inspection rendered valid his grievances, revealing his weight of 176½ pounds, height of 69¾ inches, span of 74¾ inches, distance from floor to symphysis pubis 37¼ inches, and from symphysis to top of head, 32½ inches. The non-erectile length of the penis was 2¾ inches and the penile diameter was one inch. Testicular dimensions were: left, 1¾×1 inch; right, 2×1¼ inches. The patient possessed prominent cheek bones (Asiatic type facies), a small thin nose, and fawn-colored skin wrinkled finely around the mouth and along the jaw angles and adjacent neck areas. There were bitrochanteric and mons venerum fat pads, and moderately pendulous breasts containing fat and other subcutaneous tissue suggestive of glandular elements. Ventrally, superficial veins were prominent over the entire thorax and both axillae.

The following family and personal antecedents might have some significance: his mother died, aged 57 years from "cancer of the rectum" and his maternal grandmother "died in her 80's from cancer of the right temple." There was no other known history of malignancy.

The patient had a "severe case of mumps" when he was approximately 10 years of age. He claimed that masturbation for the first time at 14-15 years of age was climaxed by an orgasm and "normal" ejaculate. Before a year elapsed, ejaculation--for some unknown reason---became impossible; yet the patient seemed well in all other respects, having masculine habitus, strength and vigor. First sexual intercourse was at the age of 18 years—"O.K., except for lack of any ejaculate." The latter condition prevailed at the time of the present consultation. The patient engaged in coitus once each six to eight weeks.

No portion of the prostate gland was detected by rectal palpation on August 27, 1946. Extreme tenderness was evinced when the supposed area of the right upper lobe was felt. One or two drops of clear fluid were expressed from the urethral meatus onto a slide. Microscopic examination disclosed only indefinable debris. The patient masturbated unsuccessfully, trying to obtain a semen specimen.

TREATMENT AND RESULTS

Sublingual administration of methyltestosterone in propylene glycol/20 per cent alcohol (25 mg. per cc.) was prescribed.¹ From August 29, 1946 to the end of January 1947, the patient consumed 100 cc. of this product.

¹ The methyltestosterone in propylene glycol was supplied through the courtesy of Dr. W. R. Bond of the Schering Corporation.

SPERMATOGENESIS FOLLOWING THE ADMINISTRATION OF ANDROGEN AND GONADOTROPIN IN A CASE OF EUNUCHOIDISM

COINCIDENTAL NEOPLASM DURING THERAPY

ROBERT M. PERLMAN, M.D.*

THE phenomenon of spermatogenesis achieved after testosterone and gonadotropin therapy in the eunuchoid individual whose previously untreated case of relatively severe hypogonadism is reported herewith is noteworthy. While receiving treatment, the patient suddenly developed a malignant tumor. Neoplastic growth—intriguing at all times—merits special consideration when it occurs rapidly in the wake of endocrine therapy in humans, even if the element of chance must be weighed heavily in the theoretical evaluation of possible etiology.

CASE REPORT



Fig. 1. 31-year-old patient with prepuberal hypogonadism—preceding therapy.

The patient (Fig. 1), a 31-year-old male American of Polish-Bohemian extraction, first examined on August 27, 1946, complained chiefly of immature and effeminate appearance, high voice, small dimensions of his external genitalia, scant growth of beard, pubic, axillary and other body

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4½ pounds. He received his last injection of A.P.L. on this day. On January 28, 1947, he complained of a mass in his right lower quadrant; it had appeared, he insisted, and grown rapidly during the last five to seven days. He felt that there was some relationship between this new growth and his work duties as a machinist, claiming undue pressure resulted against his right lower abdominal wall when the routinely applied heavy wrenches to various pipes. Inspection disclosed a subcutaneous tumor mass measuring approximately three inches in length and elevated one inch above the abdominal wall surface, located along the lateral margin of the right rectus muscle, about 1½ inches below the umbilicus (Fig. 2). Two possible diagnoses were considered: 1) atypical hernia, and 2) neoplasm. Surgical removal of a hematoma-like, encapsulated mass was performed on February 4, 1947 (Fig. 3).



FIG. 2. Showing right lower quadrant mass following surgical preparation.

When first seen by the pathologist, the specimen consisted of a cystic or pseudocystic structure $3.5 \times 3.5 \times 2.5$ cm. in size, possessing a capsule composed of smooth, glistening sections alternating with areas of fibrous tags. The fibrous wall, raggedly lined, was 1 to 1.5 mm. thick. There were other numerous irregular fragments of tissue which had been dissected away from within the cavity. Some were relatively soft, friable, yellowish, glistening and homogenous; others were frankly hemorrhagic. One portion presented an organizing hematoma.

Sections were examined microscopically by the attending pathologist and two consultants (Fig. 4). The respective diagnoses were:

He also applied 100 grams of ointment (2 mg. methyltestosterone per gram) percutaneously to the external genitalia, abdomen, chest and face. Therapeutic consequences were progressive almost immediately and dramatic in the positive sense. On September 28, 1946, one month after the beginning of treatment, the patient enthusiastically reported marked amelioration of his initial symptomatology. Objectively, he had gained 11½ pounds. Penile dimensions were moderately increased. His voice seemed lower. On October 26, 1946, the patient elatedly recounted that he had ejaculated successfully shortly after his visit on September 28, 1946. Clinically, there was notable increase in masculine hair growth and musculature. The breasts seemed masculine in contour and consistency. A further weight gain of 4½ pounds was recorded. On November 5, 1946, masturbation yielded a specimen for semen analysis performed on hanging drops and fixed smears.² The diagnosis was *aspermia*. Consequently, it was decided to give the patient a series of gonadotropin injections, hoping thereby to stimulate further testicular growth, function and possibly, spermatogenesis.

An initial dose of chorionic gonadotropin in the form of 1.0 cc. of A.P.L. (1,000 I.U. per cc.)³, injected intragluteally, was followed by an injection of 0.75 cc. every second to third day thereafter until the fifteenth injection, when subsequent doses were reduced to 0.5 cc. A total of 24 injections was given, terminating on January 20, 1947. Perineal "soreness and waves of pain" through the perineum, testes and penile shaft followed several of the injections by a matter of four to six hours, persisting in a few instances until the next day. After the ninth injection, the patient remarked about his progressively increasing stamina, general sense of well being and rapidity of beard growth. By November 23, 1946, there was another weight gain of 5 pounds. The fawn-colored, finely wrinkled facial skin had assumed normal texture and complexion. Cheek bones were no longer prominent. Physical contours appeared masculine. Examination on December 20, 1946, revealed 27¾ pounds gained since therapy was started. Non-erectile penile length was 4½ inches and the penile diameter was 1½ inches. The testes measured: left, 2½ × 1¾ inches; right, 2½ × 1½ inches. There was increased axillary and pubic hair, and new hair growth over the sternum, lower abdomen, dorsum of hands, arms and, especially, both thighs.

The peak of the patient's total weight gain of 31½ pounds was charted on January 13, 1947. One week later, on January 20, 1947, he had lost

² All semen examinations in this case were performed by Dr. Gerson R. Biskind.

³ The A.P.L. was supplied through the courtesy of Ayerst, McKenna & Harrison, Ltd., Rouses Point, N. Y.

1) "*Mesothelioma (malignant)*. The most likely site of origin would seem to be a portion of isolated serous membrane along the spermatic cord. . . ."

2) "*Fibrosarcoma with pritheliomatous pattern*, probably originating in fascial tissues . . . nature . . . not entirely clear . . . no normal structure



FIG. 5. Appearance of patient three weeks following surgical excision of tumor.
Note extent and rapid rate of pubic hair regrowth.

except . . . bit of distorted muscle and s.c. tissue to suggest the location or origin . . . cellularity and undifferentiated appearance of cells suggest . . . tumor is highly malignant and will undoubtedly recur. . . ."

3) "*Moderately malignant endothelioma, inguinal region (diffuse endothelial sarcoma)*." Mentioning possible sites of origin, this consultant included peritoneum, bursae, joints, but felt that the tumor cells definitely did not appear to be of testicular, spermatogenic or Leydig cell origin, and



FIG. 3. Surgically exposed tumor mass *in situ*.

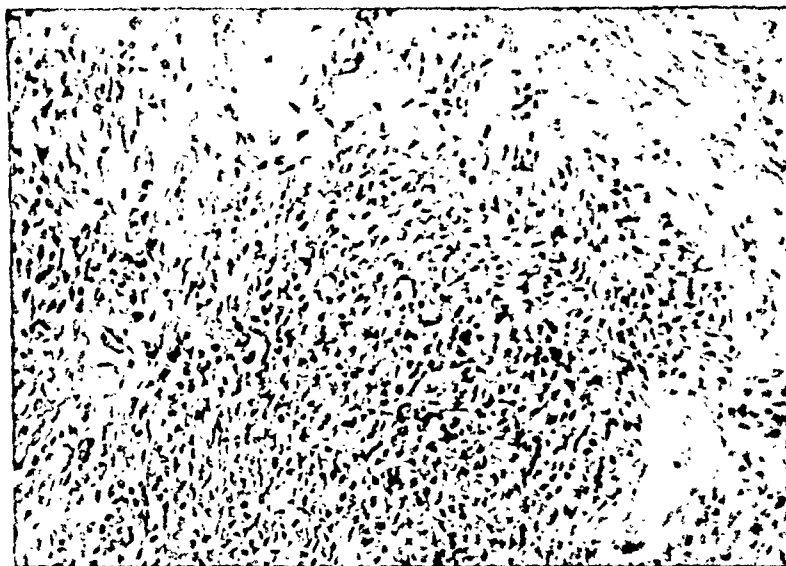


FIG. 4. Microscopic section from tumor area showing closely packed spindle-shaped cells with moderately vesicular nuclei; hematoxylin-eosin stain; Zenker fixation; $\times 100$.

SUMMARY

A 31-year-old male with a clinical picture of prepuberal hypogonadism responded favorably, from the viewpoint of masculine status, to androgen and gonadotropin therapy. Especially significant was the later-proven spermatogenesis in this individual who was unable to ejaculate prior to treatment. During therapy a mass developed in the right lower quadrant and was excised surgically. Microscopic analysis proved it to be a malignant tumor. Possible etiologic factors are discussed briefly. The factual recording of this interesting sequence of events helps demonstrate anew the enigma surrounding the relationship of endocrines to neoplastic growth; moreover, it lends material bulk to the increasingly popular protest against hasty, unscientific tendencies to ascribe over-all, antispermatogenic properties to the androgens.

Acknowledgment

The author hereby expresses deep gratitude for special services rendered by Dr. Sanford E. Leeds (surgeon), Dr. Gerson R. Biskind (pathologist), Dr. Joseph Levitin and Dr. Helen B. Weyraud (roentgenologists).

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that myoblastic or muscle origin also seemed extremely unlikely.

The patient returned periodically for subsequent endocrine follow-up. Androgen and gonadotropin therapy, ordered discontinued when the tumor mass was discovered, were not recommenced. On February 26, 1947 (Fig. 5), his weight was $21\frac{1}{2}$ pounds less than the maximum noted on January 13, 1947. On March 7, 1947, he had regained $2\frac{1}{2}$ pounds but a new subcutaneous mass, $3\frac{1}{2} \times 2\frac{1}{2}$ inches, seemed fixed beneath the recent operative scar. A course of roentgen therapy was prescribed, the patient receiving a total dosage of 3250 r to the right lower pelvis. On March 20, 1947, *two months following cessation of endocrine therapy, and after the recommended amount of X-ray treatment to the R.L.Q.*, the patient's prostate gland was definitely palpable, soft, and abnormally tender in all of its component parts. Another semen examination was requested. The following results were reported: total volume, 4.5 cc.; consistency, viscid; sperm count, 12,500,000 per cc.; motility, 10 per cent; abnormal forms, 30 per cent.

DISCUSSION

A flimsy structure, based principally on data derived from a small number of inconclusive animal experiments and inconsistent clinical experiences with humans, has been erected to support the unwarranted contention that testosterone affects adversely the seminiferous tubules of the testes and the process of spermatogenesis. Kinsell (1) recently dealt a severe blow to this structure when he reported spermatogenesis resulting in a "pan-hypopituitary" eunuchoid man from the administration of testosterone following a *supposedly proven, noneffective course of gonadotropins*. Walsh, Cuyler and McCullagh (2) and Simpson and Evans (3) were cited by Kinsell as experimental contributors to the thesis that androgens can stimulate intact germinal epithelium of the seminiferous tubules to the point of active spermatogenesis.

Attempts to explain the malignancy occurring in this case should include the ensuing considerations. The marked anabolic properties of methyltestosterone might have provided a stimulating impetus to a potentially malignant process temporarily dormant only as the result of metabolic deficiency. The A.P.L. might have been an additional stimulus, especially if the new growth derived from a terrain (perhaps embryonic cell rests) originating from the same primitive tissue layers as the gonads or other important endocrine structures. Too, the element of chronic irritation or trauma cannot be ignored. Each of the foregoing entities, alone or in combination with any or all of the others, might be of some etiologic significance. *Lastly, the possibility is manifest that the tumor occurred simultaneously with endocrine therapy purely as the result of chance.*

duce appreciable radiation effects on tissue or to disturb the iodine metabolism of the test subject. One millicurie of I^{131} (which is by definition that quantity undergoing 3.7×10^7 disintegrations per second) weighs only 0.008 microgram (11). Quantities of 0.1 to 1,000 microcuries have been used for single physiologic experiments. Often a small quantity of 5 to 100 micrograms of sodium or potassium iodide is added as a carrier to the solution containing radioiodine. This is done for several reasons, one being to minimize the loss of the isotope by adsorption on glassware or other instru-

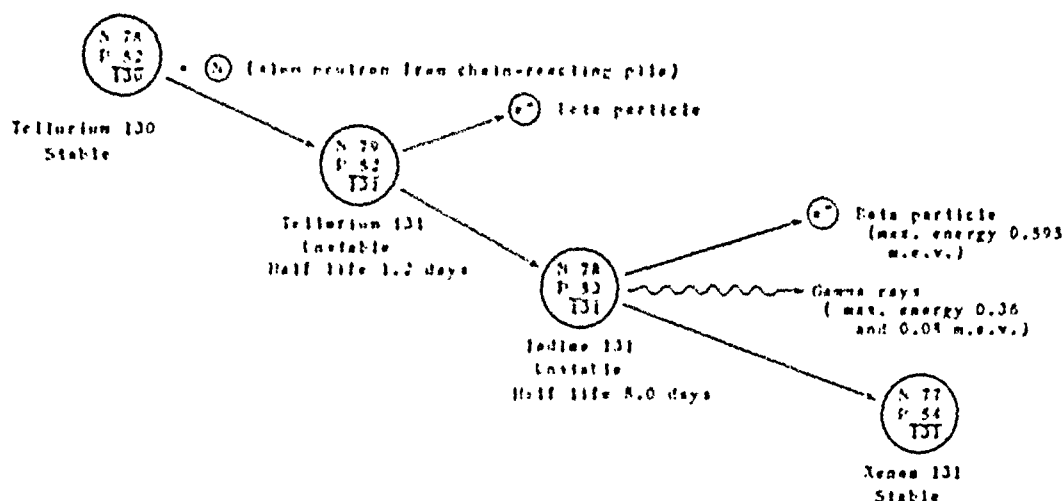


FIG. 1. The source and fate of iodine 131 . The nuclear structure of each atom is represented. N refers to the number of neutrons in each atom; P refers to the number of protons; the sum of the two represents the mass number for each atom. The diagram is simplified in that each atom of tellurium 131 disintegrates by isomeric decay, which is not shown.

ments. It has not been established that this procedure is necessary, and many investigators have used carrier-free radioiodine without difficulty.

Radioiodine has a heavy homologue, astatine (formerly called Eka-iodine or element 85), which has a half-life of 7.5 hours, and emits alpha particles. Physiologically it behaves somewhat like iodine, but its uses have not been thoroughly explored (12).

FATE OF RADIOIODINE IN THE BODY

Gastro-intestinal tract.—By use of the Geiger counter Hertz, Roberts and Evans (3) and Hamilton (4) found that ingested radioiodine is rapidly absorbed in the stomach and can be detected in the human hand within three to six minutes. Absorption in the gastro-intestinal tract is 75 per cent complete in one hour and apparently complete in three hours. The presence of food in the stomach delays absorption (13). Some of the dose absorbed from the stomach is probably secreted back into the stomach,

Endocrine Review

RADIOIODINE IN THE STUDY AND TREATMENT OF THYROID DISEASE: A REVIEW

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DURING the past decade many important advances have been made in our knowledge of thyroid physiology and disease. Radioiodine has been one of the important agents used in bringing about these achievements. Although only a few institutions, all of them in America, have used radioiodine extensively, a large literature has accumulated on the subject. In 1938, within four years after the preparation of the first artificial radioactive isotopes by Joliot and Curie (1) and the preparation of radioiodine by Fermi (2), Hertz, Roberts and Evans (3) in Boston and Hamilton (4) in California had made reports on the use of radioiodine in the study of human physiology. In this review we hope to bring together most of the important work which has been done in this field. Attention is called to the valuable review already published by Rawson and McArthur (5), which covers much of the application of radioiodine to thyroid physiology prior to 1947.

RADIOISOTOPES OF IODINE

About fourteen radioactive isotopes of iodine have been identified but only four have been used in biologic studies. The original investigators (3, 4, 6) used I^{125} , which has a half-life of twenty-five minutes and allows only the briefest experiments. By 1940, I^{130} with a half-life of 12.6 hours, I^{126} with a half-life of thirteen days and I^{131} with a half-life of eight days had been used (7) (Fig. 1). Because it is most available, I^{131} is now used almost universally. I^{131} disintegrates to xenon, each atom of iodine emitting a beta particle and two gamma radiations. The maximal energy of the beta particles is 0.595 MEV (million electron volts) while that of the gamma rays is 0.360 MEV (8). I^{131} is prepared at Oak Ridge National Laboratories, by bombardment of tellurium with neutrons in the chain-reacting pile. As is characteristic of the radioactive isotopes (9, 10), radioiodine acts chemically and physiologically exactly as does its stable isotope I^{127} , except for its radiation effects. With a Geiger counter (Fig. 2) or by autoradiography, quantities can be detected which are too small to pro-

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that radioiodine disappears from the blood slowly in myxedematous patients and rapidly in hyperthyroid patients (Fig. 3). After seventy-two hours practically all of the ingested radioiodine in the blood of patients with exophthalmic goiter has become protein-bound (Fig. 4). Perlman, Chaikoff and Morton (16) suggested that radioiodine apparently diffuses passively into and out of the intercellular fluid spaces since the maximal levels in the tissues were reached at about the same time as the maximal level in the blood.

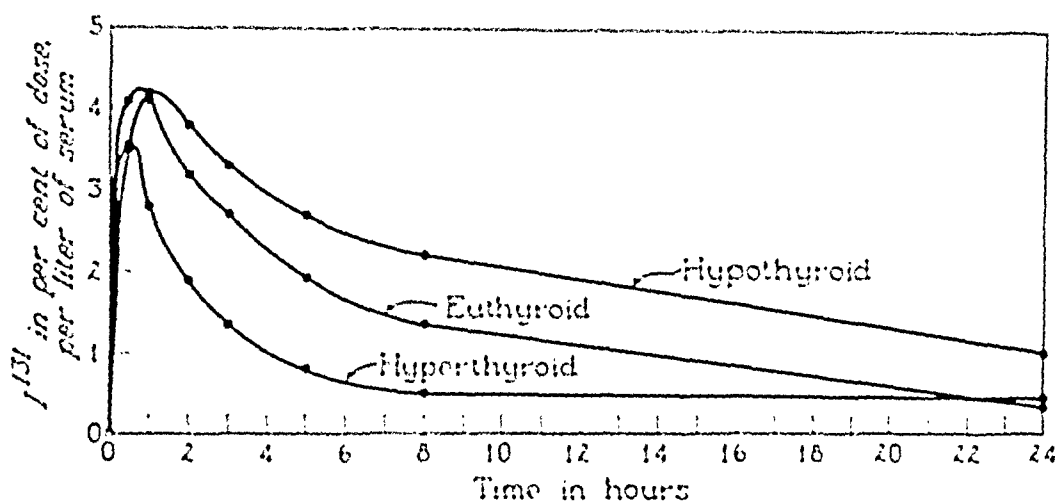


FIG. 3. Composite curves of radioiodine in serum in various thyroidal states. Radioiodine disappears from the blood more slowly in myxedematous patients than in normal subjects. It disappears from the blood more rapidly in hyperthyroid patients than in normal subjects.

Body tissues.—The original studies with radioiodine revealed that a large part of an ingested dose of radioiodine is collected by the thyroid. This will be discussed at some length in the section on metabolism of radioiodine by the thyroid. Other tissues participate to a slight extent in the collection of radioiodine. About 10 to 15 per cent of a dose of radioiodine is not accounted for by thyroidal collection and urinary excretion (18). Loss of radioiodine by technical error, by exhalation in expired air, by secretion in sweat and by excretion in feces may account for a part of this fraction of the dose but the greatest part of it probably is collected by various body tissues. Small amounts of radioiodine have been demonstrated in various organs of animals to which radioiodine had been administered, particularly in the ovaries, pituitary, lungs, kidneys and liver (19, 20). The parathyroids were not found to collect appreciable quantities of radioiodine (21). Rall and co-workers (22) measured the collection of radioiodine by various body tissues in the case of a patient who died fifty-seven hours after receiving 63 millicuries of I^{131} for treatment of advanced car-

since the stomach and salivary glands have been shown to secrete radioiodine in fairly high concentration when it is injected intravenously (14). Only a small part of a tracer of radioiodine appears in the stool, usually not more than 3 per cent and never more than 11 per cent (13, 15, 16).

Extracellular fluids.—McConahey and co-workers (17) found that concentration of radioiodine in the blood rose to a maximal level within an



FIG. 2. Measurement of radioiodine in the thyroid with Geiger counter. The counter is heavily shielded in lead. The shield has a window which is covered by a metal filter through which gamma rays, but no beta particles, may enter the counter tube. Radiations detected by the tube are counted automatically by the scaler on the right. The thick lead plug is placed over the window of the shield so that counts may be made to correct for radiation entering the tube from sources other than the target. Quantitative estimations of radioiodine in vivo are not yet very accurate.

hour after ingestion of the tracer. The curve of total radioiodine in serum plotted against time after oral administration of a dose was shown to have three components: 1) an initial rapid increase followed by a brief rapid fall, which is regarded as reflecting gastro-intestinal absorption and equilibration with body fluids; 2) an exponential fall, which is regarded as reflecting disappearance of radioiodine as iodide from the blood into the thyroid, the urine and other sites of disposal; 3) a phase which represents the appearance in the blood of organically bound radioiodine. They showed

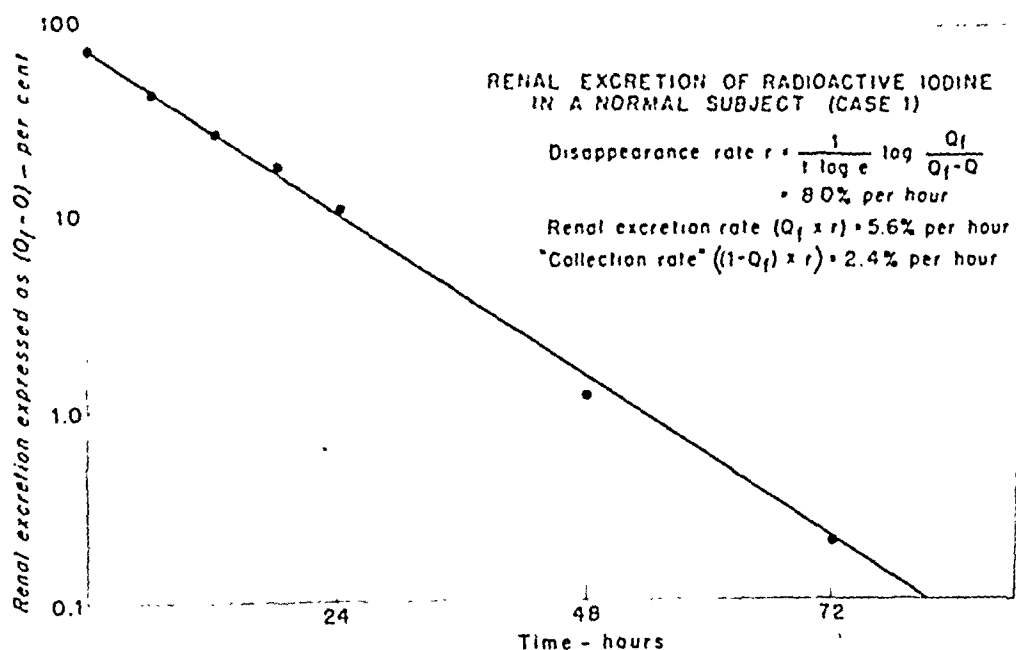
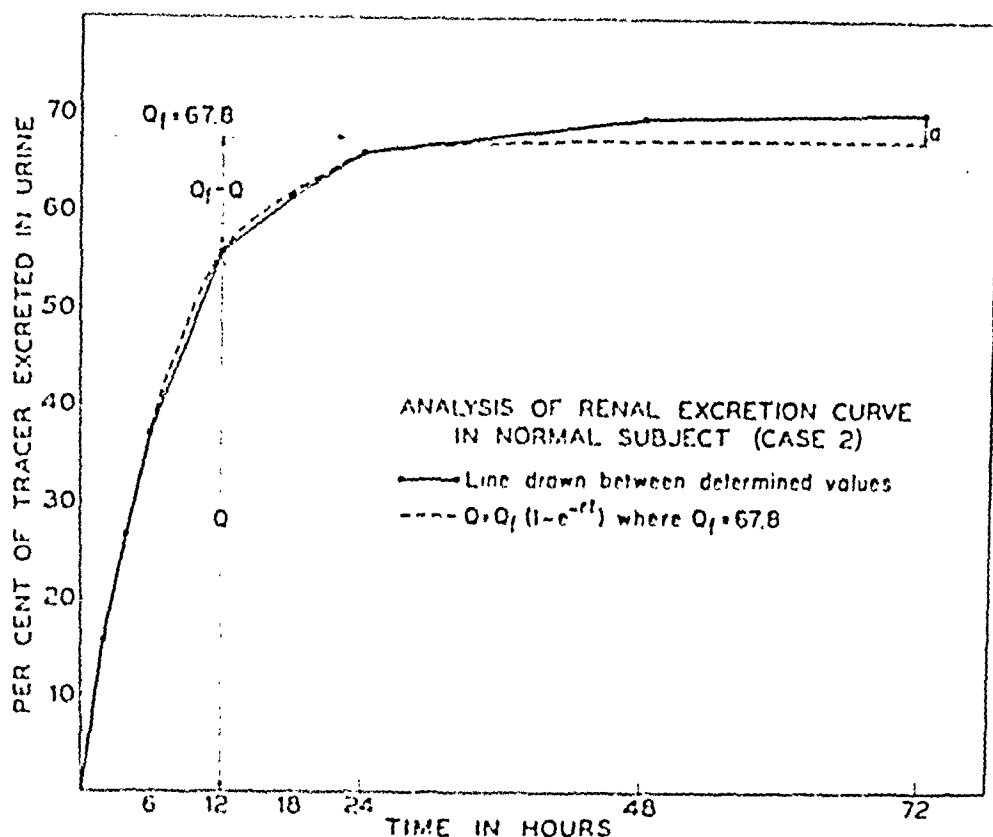


FIG. 5. Analysis of urinary excretion of radioiodine in a normal subject. A. The quantities employed in analyzing the curve: Q_t is the asymptote or plateau which the initial excretion curve approaches; the quantity $Q_t - Q$ may be plotted as a straight line on semilogarithmic paper. B. The form of the renal excretion curve when plotted as the expression $Q_t - Q$. From the line the value r , or disappearance rate, may be determined. (Modified from Keating, Power, Berkson and Haines (24).)

cinoma of the thyroid. The following tissues collected radioiodine: thyroid (22 per cent of dose), thyroid malignant tissue 1.2 per cent, muscles 3.3 per cent, liver 3.9 per cent, brain 2.0 per cent, lungs 0.6 per cent and kidneys 0.1 per cent.

Urinary excretion.—Hamilton and Soley (23), as well as others (3, 7) have pointed out that a large part of an ingested dose of radioiodine is excreted in the urine within forty-eight hours, after which time only

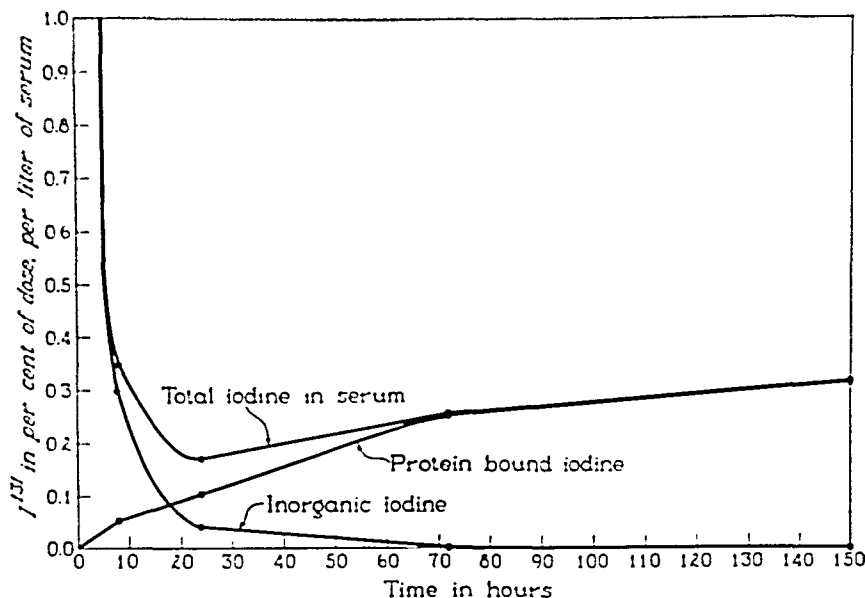


FIG. 4. The appearance of protein-bound radioiodine in the blood of a patient with exophthalmic goiter who had received a therapeutic dose of radioiodine, expressed in per cent of dose per liter of serum. The radioiodine in the serum gradually becomes protein-bound, so that at seventy-two hours virtually all the radioiodine in the serum is protein-bound and none is inorganic iodide. (From McConahey, Keating and Power (17).)

minute quantities are excreted. Keating, Power, Berkson and Haines (24) studied the urinary excretion of radioiodine using a tracer dose of 100 microcuries of I^{131} with a carrier of 100 micrograms of sodium iodide. Urine was collected at frequent intervals for seventy-two hours after administration of the dose. Knowing that all but 10 or 15 per cent of the ingested dose which was not excreted in the urine was collected by the thyroid (23), they were able to use the urinary excretion figures as an indicator of the approximate collection by the thyroid. By plotting cumulative urinary excretion against time, they obtained an exponential curve from which were estimated four factors: 1) a renal fraction (that part of the dose of radioiodine asymptotically excreted in the urine); 2) a dis-

iodide free of protein linkage is collected selectively by the thyroid (6). Keating and co-workers (24), on the basis of calculations of the "accumulation rate," have suggested that the normal rate of iodine uptake by the thyroid is about 2 to 4 per cent per hour of the normal total iodide present in the blood and body fluids. Hamilton (12), Hamilton and Soley (13, 23) and Childs and co-workers (26) have shown by tracer studies of radioiodine with varying sized carriers of sodium iodide or potassium iodide that small doses of 100 micrograms or less of natural iodine, I^{127} , are handled efficiently by the thyroid, which collects about 20 or 30 per cent of the

TABLE 2. VALUES FOR RATE CONSTANT r DETERMINED FROM URINE, BLOOD, THYROID AND THIGH*

Condition	Cases	r (per cent per hour), mean†				Variability of r ‡	
		Urine	Blood	Thyroid	Thigh	S.D.‡ variance	P
Euthyroid	6	14.5 ± 1.1	15.0 ± 0.8	12.9 ± 1.1	14.0 ± 1.0	3.76	>0.05
Hyperthyroid	7	39.0 ± 5.3	39.3 ± 5.5	40.6 ± 6.6	37.0 ± 5.1	26.81	>0.05
Hypothyroid	4	6.0 ± 0.5	8.5 ± 1.3	6.0 ± 0.7	6.9 ± 0.5	2.68	>0.05

* From: Lucien T. J., Keating, F. R., Jr., Williams, M. M. D., Berkson, J., Power, M. H. and McCoubrey, W. M. (18)

† The value after the \pm is the standard error of the mean.

‡ The variability of r for each case was calculated as the variance (squared S.D.) of the four estimates derived (urine, blood, thigh and thyroid); the value in the table is the mean variance for the group. The P value was calculated by the method of analysis of variance to determine whether the differences among the determined r 's were significant statistically. A value of $P < 0.05$ is generally considered a good criterion of significance.

ingested dose within forty-eight hours. Larger doses, such as several milligrams, saturate the iodine capacity of the thyroid and are only partly as well collected (12, 26) (Fig. 6).

This accumulation of iodine by the thyroid may be divided into two components, the trapping of inorganic iodide, and the conversion and storage of organically bound iodine. Trapping is the rapid concentration by the thyroid of relatively large quantities of circulating iodide presumably in the follicle cells. If the iodide trapped by the thyroid cannot be utilized for hormone synthesis most of it passes out of the gland within twenty-four hours. Simultaneous with trapping in the normal thyroid, the trapped inorganic iodide apparently is being converted to organically bound iodide and accumulated in the form of diiodotyrosine and thyroxine in the colloid. This converted iodine accounts for more than 90 per cent of the iodine collected by the thyroid forty-eight hours after a tracer dose has been ingested and is thus responsible for the greatest part of the thyroid's ability to collect iodine. These assumptions are based on the following studies with radioiodine.

Collection of iodine by the thyroid has been studied mainly by two types of experiments: autoradiography and biochemical determinations.

appearance rate (the proportional rate of disappearance of radioiodine from the blood); 3) a renal excretion rate (the proportional rate of excretion into the urine obtained by taking the product of renal fraction and disappearance rate); 4) a "collection rate" (or more properly, extrarenal disposal rate), defined as the proportional disappearance into other sites than the kidneys, of which the most important is the thyroid, obtained by taking the difference between disappearance rate and excretion rate (Fig. 5 and Table 1). In normal persons the kidneys excrete about 60 to 70 per

TABLE 1. ANALYSIS OF URINARY EXCRETION OF RADIOIODINE IN 169 PERSONS*

	No. of cases	Basal metabolic rate, per cent	Renal fraction per cent of dose	Disappearance rate, per cent per hour	Renal excretion rate, per cent per hour	Collection rate, per cent per hour
Normal volunteers	4	- 1 \pm 2.0	65.2 \pm 2.5	11.1 \pm 1.8	7.2 \pm 1.0	3.9 \pm 1.4
Euthyroid patients with carcinoma of thyroid	28	0 \pm 2.4	64.5 \pm 1.9	10.9 \pm 0.7	6.4 \pm 0.4	3.9 \pm 0.6
Adenomatous goiter: without hyperthyroidism with hyperthyroidism	26	+ 1.8 \pm 1.8	62.1 \pm 3.8	13.1 \pm 4.8	7.3 \pm 0.5	5.7 \pm 1.1
	23	+25.8 \pm 2.0	43.6 \pm 3.3	16.3 \pm 1.9	6.2 \pm 0.8	10.1 \pm 4.1
Exophthalmic goiter	64	+39.0 \pm 1.6	23.4 \pm 3.9	30.0 \pm 3.3	5.5 \pm 0.4	24.5 \pm 1.9
Myxedema	24	-27.7 \pm 0.8	85.5 \pm 0.6	6.3 \pm 0.4	5.4 \pm 0.4	0.93 \pm 0.11

* Study of the cumulative urinary excretion of a tracer dose of radioiodine has provided valuable information on the kinetics of iodine metabolism. From these excretion curves the fraction of the tracer dose excreted by the kidneys, the disappearance rate of radioiodine from the blood, the rate of renal excretion and the rate of collection by the tissues (the thyroid principally) can be determined. This table shows what these factors are for normal subjects and for patients with thyroidal disease.

cent of the administered dose within forty-eight hours, at a rate of about 5 to 9 per cent of the circulating radioiodine per hour. The rate and amount of renal excretion are diminished when there is impaired renal function. The calculations of disappearance rate have been strikingly uniform whether based on renal excretion curves (24), thyroid uptake curves measured by a Geiger counter (18), curves of radioactivity over a peripheral part of the body (thigh) (18) or curves of disappearance from the blood measured directly from samples of blood (17) (Table 2).

METABOLISM OF IODINE BY THE THYROID

Vanderlaan and Vanderlaan (25) present the metabolism of iodine by the thyroid as a three-phase process. The first phase involves iodine accumulation; the second involves hormone synthesis and the third involves the secretion of hormone.

Iodine accumulation.—As soon as radioiodine enters the blood stream, the thyroid begins to collect it (3) and the kidneys begin to excrete it, the two organs competing for the available iodine according to their capacities to handle it (24). Hamilton (12) pointed out that the thyroid has the capacity of concentrating iodine to 10,000 times the blood level. Probably only

loid, it was suggested that the cells convert inorganic iodide into organically bound iodide—diiodotyrosine and thyroxine—which is stored in the colloid.

Chagas, de Robertis and Couceiro (41), using micropuncture and aspiration of colloid directly from the follicles of the thyroid of rats, found radioiodine appearing in the colloid within thirty-five minutes and reaching a peak concentration within forty hours after radioiodine had been injected into the animals. Mann and Leblond (42), by fractionation studies on thyroids of dogs given radioiodine, concluded that the iodide coming from the blood stream accumulates in the thyroid mainly as diiodotyrosine; in their opinion the diiodotyrosine fraction is the natural precursor of thyroxine. Lein (43) separated the radioiodine collected by thyroid glands of rabbits into acetone-soluble and acetone-insoluble fractions. Acetone-insoluble radioiodine was found in small quantities as early as five minutes after the injection and increased rapidly. Lein expressed the opinion that as fixed (trapped) iodine is converted to protein-linked forms, the previously saturated "fixing mechanism" (trapping mechanism) may renew its capacity to collect circulating iodine.

It remained for Vanderlaan and Vanderlaan (44) and Taurog, Chaikoff and Feller (45), working independently, to prove that the trapping by the thyroid of inorganic iodide is a process distinct from conversion to, and accumulation of, iodide in organic form. Vanderlaan and Vanderlaan found that the thyroid glands of rats in which hormonal synthesis is blocked by propylthiouracil (see section on Antithyroid Drugs) are still capable of concentrating (or trapping) large quantities of iodine when potassium iodide is injected. This iodine is held in the reduced form as inorganic iodide. Thiocyanate, which inhibits the collection of iodide by the thyroid, was shown to cause rapid discharge of the trapped inorganic iodide, but did not release organically bound iodide.

Taurog, Chaikoff and Feller (45) presented almost identical results: thyroids of rats made goitrous by feeding propylthiouracil concentrated injected iodine despite a complete block in synthesis of organic iodine compounds. Radioiodine entered and left the thyroid to form a curve which paralleled that of radioiodide in plasma, suggesting an equilibrium between the two. Both curves depicted a maximal concentration within one half hour and fell rapidly thereafter so that nearly all the tracers had left the thyroid after twenty-four hours. The trapped iodine, which was taken up by the thyroid and discharged from it within twenty-four hours, is thought to be in inorganic form or possibly bound to protein by a labile linkage.

Hormone synthesis.—The thyroid is said to contain iodine in only three

Hamilton, Soley, Reilly, and Eichorn (27, 28) made autoradiographs by exposing photographic film to radiations from microscopic sections of thyroid tissue of patients who had received radioiodine. By developing the film and superimposing it on the stained sections they were able to study the localization of radioiodine in thyroid tissue. Leblond and co-workers, (29-34) as well as Evans (35) and Marinelli and others (36-40) have made several studies with autoradiography. By the use of thyroid tissue from

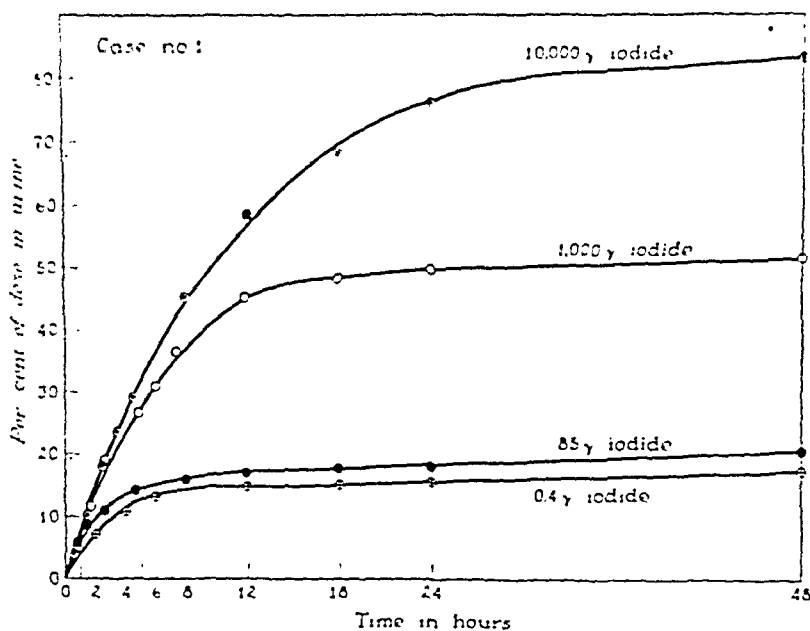


FIG. 6. Repeated tracers in the same patient showing the effect of varying the quantity of iodide in the dose as carrier. Quantities of 100 micrograms or less behave essentially alike, while larger amounts show progressively less accumulation of iodide by the thyroid. (From Childs, Keating, Williams and Power (26).)

rats (31) and from human beings (34) radioiodine was found to accumulate rapidly in, and spread diffusely through, the colloid in a stable form which was not washed out by alcohol or the usual fixation processes. This was thought to be organically bound iodide and was found in the colloid as early as thirty minutes after administration. Less than 10 per cent of the radioiodine taken up by the gland was in the inorganic form, the rest being incorporated in diiodotyrosine or thyroxine. No radioiodine was observed in the follicular epithelium. Later Gross and Leblond (30) found radioiodine in the epithelium as well as in some of the follicles of rat thyroids one-half hour after administration of the dose. Since after an interval of twenty-four hours the radioiodine was found almost exclusively in the col-

workers (51), using radioiodine with in vitro studies, found an exchange of iodide ions between iodine and diiodotyrosine at a pH of 4 to 5.5. Another bit of interesting information is the evidence of formation of thyroxine and diiodotyrosine in completely thyroidectomized animals. In rats several months after thyroidectomy I^{131} tracer studies revealed newly formed radiodiiodotyrosine and radiothyroxine. As early as ninety-six hours after injection 30 per cent of the I^{131} contained in the liver and

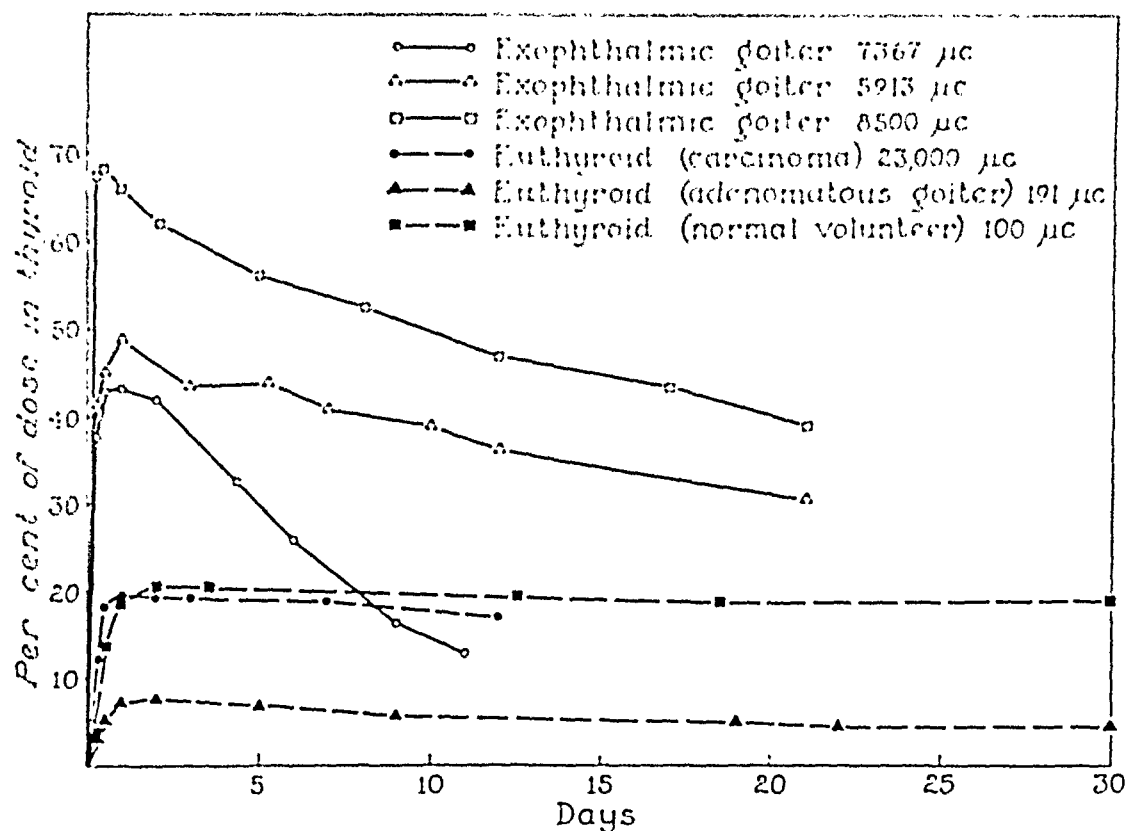


FIG. 7. The secretion of radioiodine previously collected in the thyroid appears to take place more rapidly in patients with hyperthyroidism than in euthyroid persons. These curves cannot be regarded as completely demonstrating this fact, since the radiation effects of radioiodine in the therapeutic dose given may have increased the rate of iodine secretion by the thyroid.

small intestine was organically bound, about 20 per cent as diiodotyrosine and 8 per cent as thyroxine (52). McConahey and co-workers (17) followed the plasma levels of inorganic and organic iodide of a myxedematous patient who presumably had no functioning thyroid and found a gradual conversion of inorganic to organic iodide during a period of several days.

Secretion of hormone.—Although the collection by the thyroid of a tracer dose of radioiodine without carrier is completed within a day or two, the radioiodine is liberated into the blood stream apparently as hormone

different chemical forms (42, 46): 1) a small proportion of trapped inorganic iodide, less than 10 per cent; 2) thyroxine, representing about 25 per cent, and 3) the remainder as diiodotyrosine. It is assumed that thyroxine and diiodotyrosine share in the formation of the protein molecule of thyroglobulin, and that diiodotyrosine is a precursor of thyroxine. The results of radioiodine studies are compatible with these views. As studies on accumulation of radioiodine by the thyroid have shown, inorganic radioiodine undergoes conversion to diiodotyrosine and thyroxine rapidly; this begins almost as soon as the thyroid collects iodine from the circulating blood (34, 42, 43). Perlman, Morton and Chaikoff (47) studied the rate at which the thyroid glands of rats and sheep given radioiodine produce diiodotyrosine and thyroxine. Of the injected radioiodine, 1.5 to 3.0 per cent was present as radiothyroxine in the rat thyroid within two hours after administration. Formation of thyroxine in the sheep occurred at a slower rate. The amount of newly formed thyroxine increased with time up to 19 per cent of the total dose of radioiodine in forty-eight hours. Even a larger amount of radiodiiodotyrosine was formed. Despite considerable fluctuation in the actual amount of radioiodine deposited as thyroxine and diiodotyrosine in the gland, the proportion of the total labeled iodine represented by each of these fractions remained fairly constant at each interval. Mann and Leblond (42) gave carrier-free doses of radioiodine to dogs and found the diiodotyrosine fraction of radioiodine in the thyroid to be relatively high (8 to 11 per cent) at one-half hour, 10 to 15 per cent at eight hours and 43 per cent at forty-eight hours. The thyroxine fraction was 0.3 to 0.4 per cent at one-half hour, 1.0 per cent at eight hours and 2.4 per cent at forty-eight hours. Considering relative specific activity there was a gradual increase to a relatively higher level of thyroxine. When dogs were given excessive doses of iodine comparable to those used as therapy in medical practice, the iodine was thought to be incorporated in the glands, probably as stored iodide. The proportion of iodine transformed to diiodotyrosine was much smaller for the larger dose than in the case of the smaller dose. Taurog and Chaikoff (48) constructed specific activity-time curves for the iodine of the thyroxine and diiodotyrosine fractions of the thyroid glands of rats that had received a dose of radioiodine. The curves satisfied Zilversmit's criteria for a precursor-product relationship (49), thus providing further evidence that diiodotyrosine is the precursor of thyroxine. Studies on thyroid slices have shown that the conversion of iodide to diiodotyrosine and thyroxine is a function of cellular organization as it does not take place in homogenates (50).

The possibility of ionic exchange of inorganic and organic iodine to confuse these results is not likely but has not been disproved. Miller and co-

thyrotropic hormone begins. Apparently, rapidly proliferating thyroid tissue has a diminished capacity to collect iodine from the blood. I^{131} uptake increases as long as cell height increases (hypertrophy), but there is no increase of I^{131} uptake with further enlargement of the gland (hyperplasia). Increase of collection occurs only after hypertrophy has taken place. Thyrotropic hormone produces prompt and early acceleration of loss of previously stored I^{131} from the thyroid. This may be a measure of accelerated secretion of thyroid hormone from the gland induced by thyrotropic hormone stimulation (59).

Cortell and Rawson (60) stated that exogenous thyroxine depresses both the amount of thyrotropic hormone available to the thyroid and the action of the thyrotropic hormone available on the thyroid. Morton, Perlman and Chaikoff (61) demonstrated that injections of thyrotropic hormone increase the amount of radioiodine which becomes organically bound in the thyroid and in the plasma. Leblond and Süe (6) and others (62) found that hypophysectomy diminishes the uptake of radioiodine by the thyroid, and Vanderlaan and Vanderlaan (25) stated that thyrotropic hormone is necessary for the synthesis of thyroid hormone.

Antithyroid drugs. Radioiodine has been a great aid in studying the action of these agents on iodine metabolism. Likewise the antithyroid drugs have aided in the study of iodine metabolism, particularly in clarifying the trapping of iodine by the thyroid (see section on Metabolism of Iodine by the Thyroid). Rabbits were found to have an increased thyroidal uptake of radioiodine when fed cabbage or methyl cyanide (7). Anaerobiosis and substances that inhibit cytochrome oxidase, including cyanide, azide, sulfide and carbon monoxide, have been found to depress the formation of radioactive diiodotyrosine and thyroxine by thyroid slices bathed with radioiodine. Apparently this function is linked with aerobic oxidations in which the cytochrome-cytochrome oxidase system is involved (63). The xanthine oxidase system may enter into the process of iodinating protein (64). Cyanide and sulfide also markedly inhibit the accumulation of radioiodine by thyroid slices while azide and sulfonamides, although preventing conversion to diiodotyrosine and thyroxine, do not prevent the uptake of radioiodine (65-67). These findings are further evidence that the thyroid has a mechanism for concentrating iodine that does not depend on the conversion of inorganic iodide to organically bound iodide.

Several studies of the effect of thiocyanate on iodine metabolism, all under different circumstances, have given apparently conflicting results. It appears that administration of a single dose of thiocyanate markedly depresses the uptake of iodine and its conversion to diiodotyrosine and thyroxine by the thyroid, while prolonged administration to the point of producing compensatory thyroid hypertrophy (thiocyanate goiter) some-

at a much slower rate (Fig. 7). Perlman, Chaikoff and Morton (16) found that at two hours after intraperitoneal injection of radioiodine into guinea pigs there was newly formed radioactive diiodotyrosine and thyroxine in the plasma. After twenty-six hours 50 per cent of the circulating radioiodine was present as radiothyroxine. The distribution of radioiodine in the organic and inorganic fractions in the tissue resembled that in the plasma (20). Mann, Leblond and Warren (46) calculated that 1.55 per cent of the thyroxine contained in a normal thyroid of a dog is formed each hour. Taurog, Chaikoff and Entenman (53), by injection into dogs of plasma of rats containing naturally biosynthesized protein-bound radioiodine, estimated a turnover of circulating protein-bound iodine each four to seven and a half hours. In their experiments with specific activity-time curves, Taurog and Chaikoff (48) estimated the rate of secretion of thyroxine by the thyroid of rats to be about 2 micrograms per 100 Gm. of body weight per twenty-four hours. Further work is needed before these results will be correlated. Johnston (54) has emphasized that measurements of stable and radioactive iodine in blood and tissue provide a ratio of specific radioactivity which is valuable in determining turnover rates of radioiodine by the thyroid.

Taurog and Chaikoff (55), by using extraction methods on plasma of rats given I^{131} , concluded that 90 per cent of plasma iodine behaves like thyroxine in solubility properties. This is offered as proof that thyroxine is the actual circulating hormone. Leblond and Gross (56) came to the same conclusion by similar experiments.

FACTORS INFLUENCING METABOLISM OF RADIOIODINE BY THE THYROID

Thyrotropic hormone.—Hertz and Roberts (57) injected thyrotropic hormone into rabbits and found an increase in the uptake of radioiodine by the thyroid, as well as an increase in follicle cell height, size of the thyroid and metabolic rate. These factors all paralleled the amount of thyrotropic hormone given except for the collection of radioiodine, which reaches a peak beyond which it cannot increase. Pretreatment with iodine lessened the effect of thyrotropic hormone in producing these changes; if administration of thyrotropic hormone was continued without iodine, exhaustion of the thyroid eventually took place and it lost its capacity to collect iodine.

Keating and co-workers (58, 59) have found, in chicks, that with increasing degrees of stimulation by thyrotropic hormone there is a progressive increase in collection of radioiodine by the thyroid until a plateau is reached. Daily injections of thyrotropic hormone cause thyroid hypertrophy (increased follicle cell height) within twenty-four hours, but iodine uptake is not increased until forty-eight hours after administration of

doses of iodide overcame the block and resulted in uptake of iodine by the gland. We have found great variability in uptake of radioiodine by human thiocyanate goiters. In some instances a low uptake when the blood thiocyanate level was high was followed by a normal, or by an abnormally high, uptake soon after the thiocyanate had disappeared from the blood.

The confusion concerning iodine uptake by thyroids treated with thiouracil seems to be resolving itself. Thyroids of patients given thiouracil in preparation for removal of exophthalmic goiters were found to collect little radioiodine (76). This was confirmed by experiments on animals (73, 77-79) and it appeared that thiouracil prevented the uptake of iodine by blocking the formation of iodinated thyroid hormone (5). In vitro studies with thyroid slices in a bath containing thiouracil had shown normal uptake of inorganic iodide by the thyroid but a failure of conversion from iodide to diiodotyrosine and thyroxine (69, 80). These relationships were clarified when it was shown that the trapping of iodine by the thyroid remains intact and is even enhanced under the influence of thiouracil while the synthesis of organic iodide is blocked by the drug (44, 45, 71).

Stanley and Astwood (81) and Williams and co-workers (82, 83) have been able to utilize uptake of radioiodine by human thyroids as a means of determining the potency of antithyroid compounds in man. Another important contribution to the knowledge of treating exophthalmic goiter has been the finding by Rawson and co-workers (84) that iodine, when given simultaneously with thiouracil, has an involuting effect on the hyperplastic gland. These authors concluded from this observation that the involuting property of iodine depended on the reduced, rather than the oxidized form.

Other factors.—Pretreatment with, or overwhelming the thyroid with, iodine causes a diminished uptake of a dose of radioiodine (7, 85, 86). An iodine-deficient diet, however, increases thyroidal iodine collection (87). Thyroid slices exposed to varying concentrations of natural iodine (I^{127}) are inhibited in their ability to convert iodine to diiodotyrosine and thyroxine when the amount of inorganic I^{127} in the medium surrounding the slices exceeds 20 micrograms (88). Recently Wolff and Chaikoff (89-91) have extended this study to rats and have demonstrated that the thyroid traps iodide but does not convert it to organic iodide as long as the serum concentration of iodine exceeds 35 micrograms per 100 cc. This phenomenon in normal animals is offered as a possible explanation for the beneficial effect of iodine in human hyperthyroidism. Administration of large quantities of iodine after a dose of radioiodine has been collected by the thyroid may hasten the urinary excretion of radioiodine from the subject (92). This is especially true if the iodine is given within twenty-four hours after the dose of radioiodine, while there is a much smaller urinary excretion of

times, but not always, results in an increase of the uptake of radioiodine by the thyroid (68).

Among the acute experiments is the study of Franklin, Chaikoff and Lerner (69). Thiocyanate depressed the uptake of radioiodine and its conversion to diiodotyrosine and thyroxine in surviving tissue slices of thyroid. Rawson and co-worker (5) reported that radioiodine uptake by thyroids of chicks and rats was depressed from one to six hours after a single dose of thiocyanate, but the block in iodine collection is not demonstrable after twelve hours. McGinty and co-workers (70) found inhibition to the collection of radioiodine by chick's thyroid after a 10 mg. dose of potassium thiocyanate to be practically gone after twenty-four hours. Vanderlaan and Bissell (71) depleted the rat thyroid of hormonal iodide by administration of propylthiouracil and then demonstrated a marked inhibition by thiocyanate on uptake of radioiodine.

Goiter caused by prolonged administration of thiocyanate was first studied with radioiodine by Rawson, Hertz and Means (72). They observed increased uptake of radioiodine by a large thiocyanate goiter in a patient having hypertension who was still under treatment with potassium thiocyanate. Development of goiter was attributed to block of thyroid hormone formation by thiocyanate and consequent lowering of circulating hormone. This led to stimulation of the pituitary to produce an excess of thyrotropic hormone, thus causing hyperplasia of the follicular epithelium. These writers expressed the belief that an excess of iodine may force the block on hormone formation and cause liberation of thyroid hormone. They suggested that thiocyanate goiter might be prevented by prophylactic doses of iodine or by substitution therapy with thyroid. Rawson, Tannheimer and Peacock (73) found increased thyroidal uptake of radioiodine in rats made goitrous by thiocyanate in drinking water. Later work by Rawson and co-workers (74) indicated that the total uptake of radioiodine by rats fed thiocyanate over a long period was about the same as that of untreated controls, but somewhat less per milligram of thyroid tissue. In the chick, potassium thiocyanate fed over a prolonged period was found to be a potent goitrogen, causing considerable increase in total uptake of radioiodine and in uptake per gram of tissue.

On the other hand Wolff and co-workers (75) reported that the iodine-concentrating capacity of the thyroid is depressed in rats treated with thiocyanate, provided high concentrations of the drug are still present in the circulation. Within sixteen to twenty-four hours after the last dose when thiocyanate is no longer demonstrable in the blood, the enlarged gland has a greater than normal capacity for concentrating radioiodine. Vanderlaan and Bissell (71) tested the effect of varying doses of iodine on thiocyanate block in rats made goitrous with propylthiouracil. The larger

quent to control of myxedema. The bulk of radioiodide eventually appeared in the urine with little in the feces. More than half of the radioactivity from the radioiodorasein appeared in the feces and smaller amounts in the urine both prior and subsequent to control of myxedema. An appreciable quantity of the radioactivity was concentrated over the liver, suggesting an intermediate stage in the metabolism of iodocasein.

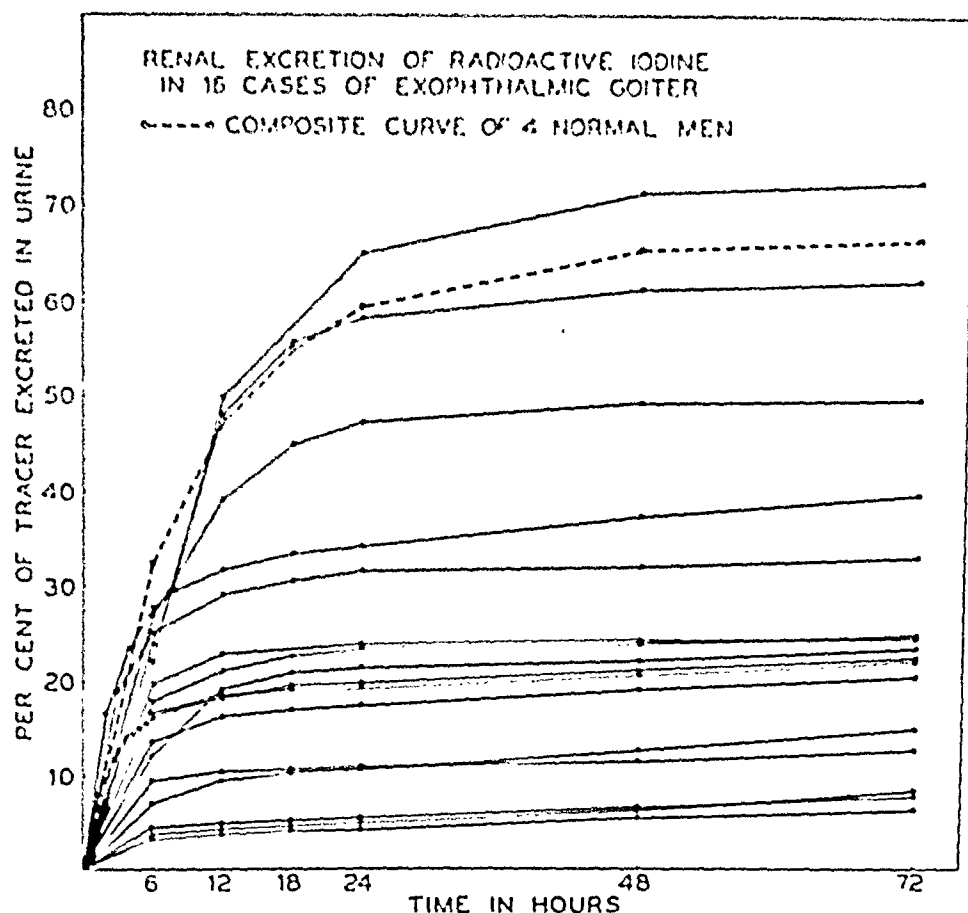


FIG. 8. Cumulative urinary excretion of radioiodine in 16 cases of exophthalmic goiter. All but 3 subjects excreted less than 40 per cent of the tracer dose. The composite normal excretion was about 65 per cent of the tracer dose. In cases of hyperthyroidism urinary excretion of radioiodine is essentially complete in a much shorter period than among normal persons. (From Keating, Power, Berkson and Haines (24).)

RADIOIODINE STUDIES IN VARIOUS THYROIDAL STATES

Hamilton and Soley (13), in their early studies with radioiodine, applied three techniques which have made the clinical use of radioiodine practical: 1) determination of urinary excretion of radioiodine, 2) determination of radioiodine uptake by the thyroid in patients by use of the Geiger counter and 3) analysis of excised thyroidal or carcinomatous tissue by autoradiography or by quantitative measurement of radioactivity.

Hyperthyroidism.—Increased iodine-collecting function of the thyroid is

radioiodine if the supplementary iodine is not given until seventy-two hours after the dose of radioiodine (93). Vitamin A deficiency in rats has been reported to cause an abnormally high level of inorganic iodide and a low level of thyroxine in the thyroid gland (94). Physiologic processes which increase the metabolic rate, including pregnancy (7) and exposure to cold (95), cause an increase in thyroidal uptake of radioiodine. Depressing the metabolic rate by exposure to warmth decreases iodine uptake (95). Administration of thyroid extract decreases uptake of radioiodine by the thyroid (96, 97).

At the present time it cannot be said with certainty that the radiation effects of radioiodine may not modify the results obtained by tracer studies. Tracer doses of 100 microcuries of I^{131} subject the thyroid to appreciable amounts of radiation. Skanse, Merrill and Evans (98) studied the effect of 0.1, 1 and 5 microcuries of I^{131} on the thyroid's growth, iodine content and response to thyrotropic hormone in cockerels. Thyroids collecting 0.1 microcurie were not altered in growth or iodine concentration. Those collecting 1 and 5 microcuries exhibited no increase of thyroid weight after stimulation with thyrotropic hormone and the 5 microcurie dose also prevented the loss of thyroidal iodine which thyrotropic hormone causes in normal animals. These doses represent radiation varying in quantity from 1,700 r.e.p. (roentgen equivalent, physical) to 8,500 r.e.p.

RADIOIODINE AS A LABEL FOR ORGANIC COMPOUNDS

Frieden, Lipsett and Winzler (99) have reported a method of preparing radiothyroxine by exchange reaction. Gross and Leblond (100) administered radiothyroxine by oral and intravenous routes to rats and noted the subsequent distribution of radioiodine in various tissues. Radiothyroxine disappeared from the blood rapidly. Within two hours more than 50 per cent of the radioiodine accumulated in the gastro-intestinal tract, liver and pancreas, mainly as thyroxine, except in the stomach where most of the radioiodine fraction appeared as inorganic iodide. Thyroidal collection was slight and lagged, indicating that there was probably conversion to inorganic form before the radioiodine was collected. At twenty-four hours, 80 per cent of the injected material was found in the feces and 11 per cent in the urine.

Hamilton, Albert, Power, Haines and Keating (15) treated a myxedematous patient with iodocasein and completely controlled the myxedema. The fate and distribution of a tracer dose of radioiodocasein were followed in this patient before and after control of myxedema and compared with the results of tracer doses of inorganic radioiodine. Distribution of inorganic radioiodine typical of myxedema was observed both prior and subse-

such as the *quantity* collected by the thyroid, they found to be relative rather than quantitative and modified by iodide disposal via urine and other tissues.

Normal values for thyroidal iodide clearance were not given; 3 euthyroid patients with nodular goiter had clearances varying from 2 to 13 cc. per minute, while 5 patients having exophthalmic goiter had clearances varying from 20 to 228 cc. per minute.

Using urinary excretion of radioiodine at the end of seventy-two hours as an indicator of thyroid uptake, Hertz and Roberts (104) found that in 23 of 29 cases more than 50 per cent of the dose was retained. Chapman and Evans (105) reported more than 50 per cent retention under similar circumstances in 11 of 12 cases. Means (106) described a diagnostic test devised by Skanse in which the urinary excretion for 48 hours after administration of a tracer dose of radioiodine varied between 6 and 32 per cent of the dose in 25 thyrotoxic patients. Keating, Berkson, Power and Haines (24) found the urinary excretion for seventy-two hours after oral administration of a tracer dose of radioiodine to be less than normal in 14 of 16 cases of hyperthyroidism (Fig. 8). Most hyperthyroid patients retained from 75 to 95 per cent of the tracer dose. Only occasionally did hyperthyroid patients retain normal quantities of radioiodine (about 65 per cent) and this finding may have been the result of previous treatment with iodine. The rate of uptake of radioiodine from the blood by the hyperfunctioning thyroid may be increased from the normal figure of 4 per cent per hour to 30 per cent per hour. Stanley and Astwood (107, 108) have used a different approach as an aid in diagnosing hyperthyroidism. First the patient is given an oral dose of a goitrogen (mercaptoimidazole) which, in their opinion, inhibits the accumulation of organically bound iodine in the thyroid. One or two hours later a tracer dose of radioiodine is given and frequent measurements with a Geiger counter are made over the neck for a few hours. In the thyrotoxic patient a greater uptake of radioiodine is observed than in the normal subject. Also discharge of radioiodine from the thyroid under these conditions is more precipitous in the thyrotoxic than in the normal subject after administration of thiocyanate. Astwood and Stanley (109) also evolved an "accumulation gradient" of radioiodine by plotting the radioactivity over the neck against the square root of time after administration of a dose of radioiodine.

These tests have become valuable in helping to establish the diagnosis of hyperthyroidism in doubtful cases. However, it must be kept in mind that renal disease, congestive heart failure (110) and thiocyanate goiter (68) may cause a low renal excretion of radioiodine. Treatment with thiouracil, thyroid, iodine, thiocyanate sometimes, ingestion of gallbladder dye, and

exhibited in hyperthyroidism by low urinary excretion of a tracer dose of radioiodine (24, 85) and by a high count of radiations over the thyroid (18, 101) (Figs. 8 and 9). Hamilton and Soley (12, 23) found a rapid and high uptake of orally administered radioiodine by the thyroids of thyrotoxic patients. When the carrier dose was large (14 mg. of sodium iodide) the high concentration of radioiodine by the thyroid diminished rapidly within a few hours, while with a small carrier (0.1 microgram) the radioiodine concentration remained high for at least several days. Hertz and co-worker (85, 102), using a small carrier dose, observed collections of radioiodine by some exophthalmic goiters to be 80 per cent or more of the dose.

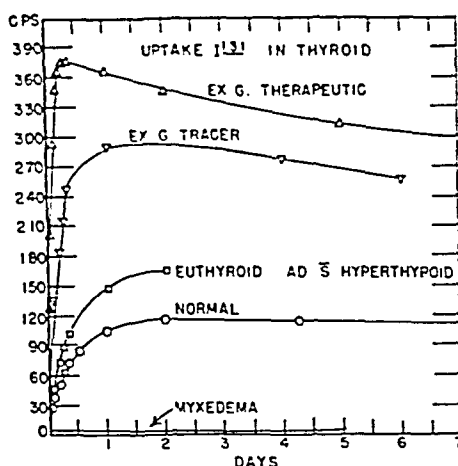


FIG. 9. Uptake of radioiodine by the thyroid measured with a Geiger counter and recorded in counts per second per millicurie dose (CPS). The uptake in exophthalmic goiter greatly exceeded the uptake in a normal subject. Uptake by an adenomatous goiter of a euthyroid subject was near normal, whereas practically no radioactivity was concentrated in the neck of a myxedematous subject.

Keating and co-workers (103) have compared various ways of measuring the iodine-accumulating function of the human thyroid. Pointing out that the thyroid collects iodide at a constant rate which is proportional to the concentration in plasma, they suggested that the most precise measure of this function is probably thyroidal iodide clearance defined as the volume of plasma cleared of iodide by the thyroid per unit time. This value is derived from serial observations of blood in addition to *in vivo* observations of the thyroid. They found that the most appropriate measure of accumulating function which could be obtained from thyroidal observations alone was the iodide-accumulation rate defined as the proportional rate at which the thyroid accumulated iodide. Other measures of accumulating function,

tissue had very little uptake and appeared atrophic, so that a hypothetical balance resulted—the normal tissue decreasing its function as the function in the nodule increased, thus maintaining a euthyroid state. When hormone secretion by a single adenoma exceeds the normal need, hyperthyroidism should result. Puppel and co-workers (114-116) reported poor iodine uptake and conversion to organic iodide in adenomas while the para-adenomatous tissue exhibited a good uptake.

Dobyns and co-workers (117, 118) have made autoradiograms of single and multiple nodular goiters and compared I^{131} collection, follicle morphology and mean acinar cell height (M.A.C.H.) of the adenomatous and extra-adenomatous tissue. In cases of adenomatous goiter with hyperthyroidism I^{131} collection and M.A.C.H. of the adenomatous tissue generally exceeded those of the extra-adenomatous tissue. In cases of exophthalmic goiter with adenomas the reverse was true. In general M.A.C.H. of adenomas could be correlated with their I^{131} collection and in one group of cases a continuous spectrum could be formed consisting at the one end of adenomas with flat cells and little collection and at the other of adenomas with tall cells and intense collection. Such "hyperfunctioning" adenomas also occurred in patients without clinical hyperthyroidism. In another group of cases very marked hyperplasia was accompanied by very little collection. This group differed from the first in having a wide distribution of acinar cell heights compared to the narrow distribution of acinar cell heights in the hyperplastic hyperfunctioning adenomas. Dobyns and co-workers expressed the belief that the nonfunctioning group formed a continuous spectrum with papillary adenocarcinoma, the follicles of which showed similar variability of cell size and similar lack of function.

Spencer and co-worker (119) studied multiple adenomatous goiters with and without hyperthyroidism. The adenomas within a single goiter collected variable amounts of radioiodine, some more, others less, than did the nonadenomatous tissue (Fig. 11). Apparently a patient who has nodular goiter will have hyperthyroidism if the total hormone produced by all the tissues with different degrees of function exceeds that required for normal metabolism.

Some diffuse goiters with or without hypothyroidism have been seen with high uptake of radioiodine and high conversion to organic iodide (28, 114-116).

Hypothyroidism.—It is not surprising that patients who have myxedema, having little or no functioning thyroid tissue, excrete about 80 per cent or more of a tracer dose of radioiodine in the urine within seventy-two hours (13, 23, 24) (Fig. 12). Geiger counts over the neck show little or no concentration of radioiodine in the region (18, 101) (Fig. 9). Exceptions may occur in certain goiters with myxedema (28) or in thiocyanate goiter with

possibly other medications may increase the urinary excretion of radioiodine to very high levels, this phenomenon persisting for varying periods after withdrawal of the medication.

Goiters.—Radioiodine uptake is almost invariably increased both in diffuse toxic goiter and in nodular goiter with hyperthyroidism (111). Hamilton and co-workers (27) stated that diffuse toxic or exophthalmic goiters collect iodine uniformly throughout the gland, but Saylor and Kelsey (112) have found a patchy distribution of radioiodine in such glands (Fig. 10).

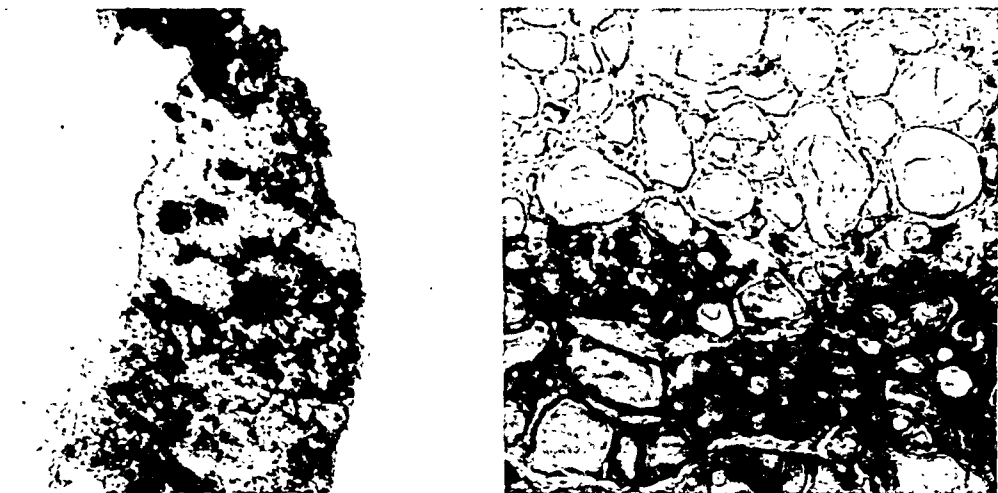


FIG. 10. Autoradiographs of a diffuse toxic or exophthalmic goiter. The goiter was removed nine days after administration of a tracer dose of 300 microcuries of I^{131} and after seven days of treatment with 30 drops of compound solution of iodine daily. There is a patchy distribution of the retained radioiodine throughout the tissue. This irregularity of retention may explain the variability of response of different thyroid glands to approximately the same dose of radioiodine. (Courtesy of Dr. Howard Saylor.)

Nodular goiters are even more difficult to understand, but the question as to whether nodules or adenomas of the thyroid function has been solved so far as uptake of iodine and its conversion to diiodotyrosine and thyroxine are concerned. Saylor and Kelsey (112) found no collection of radioiodine in adenomas within diffuse toxic or exophthalmic goiters while the non-adenomatous tissue showed good collection. Cope, Rawson and McArthur (113) studied patients who had hyperthyroidism and single adenomas of the thyroid. One patient had great uptake of iodine in the adenoma while the nonadenomatous tissue was atrophic and collected iodine poorly. In euthyroid persons who had single adenomas varying degrees of uptake were seen in the adenomas. Some collected more than the uninvolved tissue, others collected less. When an adenoma had high uptake, the uninvolved

(125) have developed a Geiger counter probe which can be cold-sterilized for use at operation in localizing thyroid tissue in an operative field made difficult by scar tissue or by retrosternal location of the goiter. Werner and Quimby (126) recently reported the use of 250 tracer doses of radioiodine in diagnostic studies of various thyroidal abnormalities. Seidlin and co-

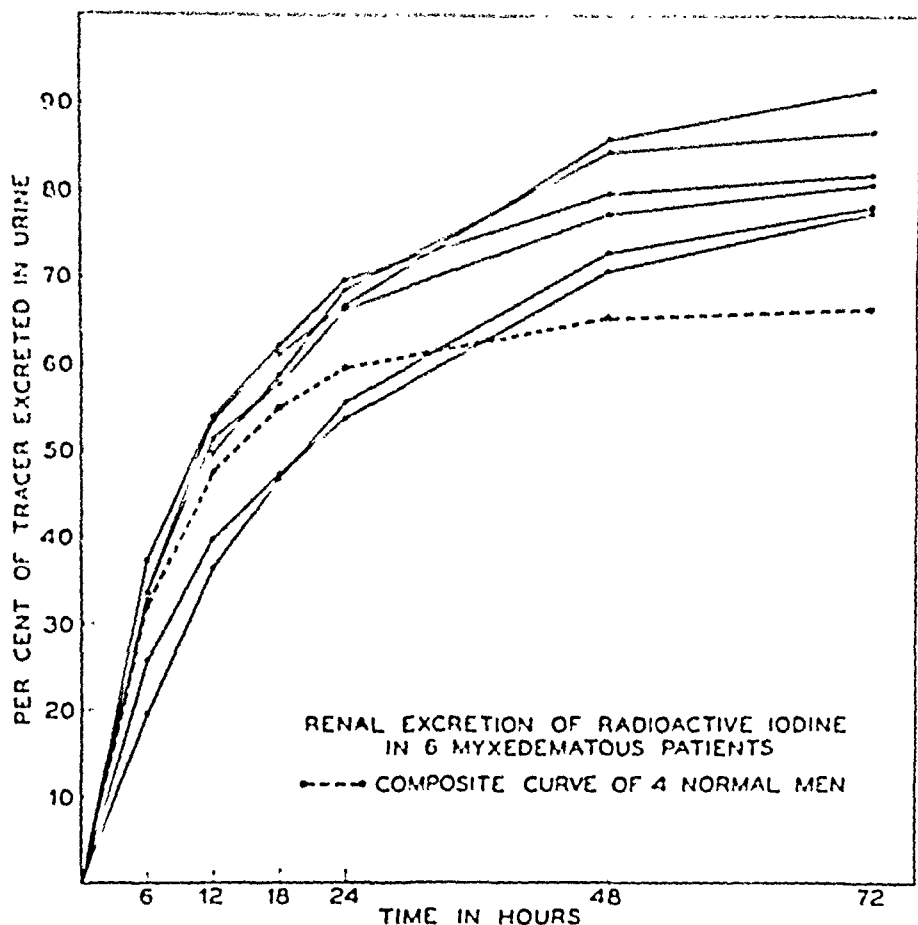


FIG. 12. Cumulative urinary excretion of radioiodine by 6 patients with myxedema. In all cases the per cent of tracer excreted in the urine exceeded that of the composite normal. The excretion curves also did not level off as quickly as did those of the normals. (From Keating, Power, Berkson and Haines (24).)

workers (127) have pointed out the value of tracer studies with radioiodine in cases in which the metabolic rate is high as a result of parkinsonism, anxiety states and heart failure. Skanse and Riggs (97) have found radioiodine tracer studies useful in differentiating spontaneous hyperthyroidism from that induced by ingestion of thyroid.

THERAPEUTIC USES OF RADIOIODINE

Internal irradiation of thyroid tissue with radioiodine affords a means of delivering a much greater amount of radiation than is possible by roent-

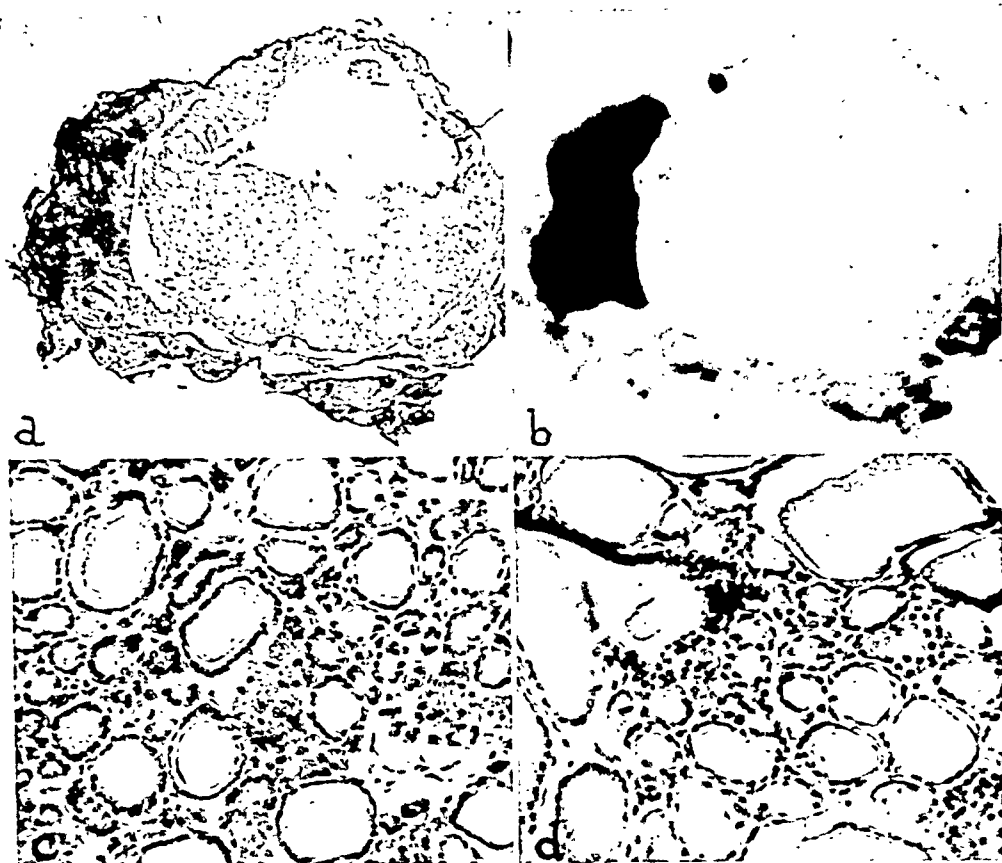


FIG. 11. Autoradiographic study of a multiple adenomatous goiter removed from a patient with hyperthyroidism. *a*. Photomicrograph of the goiter showing several adenomas. *b*. Autoradiograph of the same section. Note variability in uptake of radioiodine, the large central adenoma collecting very little, some smaller peripheral adenomas collecting much, and the nonadenomatous tissue in the lower part of the section collecting a moderate amount. *c*. Photomicrograph of the adenoma at the left of the section. *d*. Photomicrograph of the large central adenoma. Although there is an extreme difference in uptake of radioiodine in the two adenomas, their microscopic appearance is almost identical. (Courtesy of Dr. J. R. Spencer.)

myxedema (72) in which the thyroid is hungry for iodine. Radioiodine uptake by the thyroid is usually diminished in thyroiditis (120) but for some unknown reason this response varies with different patients (111). Elderly normal subjects are said to have a diminished rate of uptake of radioiodine (121).

Other diagnostic tests.—Radioiodine has also been used to determine whether intact tissue or tumor mass is thyroidal in origin (122), whether a complete thyroidectomy has been performed (123) and to discover distant metastatic growths of thyroidal carcinoma (124). Hayden and Corrigan

remaining still appeared hyperplastic, indicating that radioiodine does not affect the original stimulus causing hyperthyroidism but acts only to destroy the overfunctioning parenchyma. Average time for remission to occur was seven to eight weeks. The blood lipids tended to rise along with fall in metabolic rate while the patient improved clinically. The larger goiters did not respond as quickly as the smaller ones. Toxic reactions seen in 6 cases were much like roentgen ray sickness. Nausea, vomiting, malaise, even slight increase of gland size and fever occurred and lasted two days at most. No subsequent ill effects or leukopenia were noted, and no malignant changes were seen in the two biopsies, one as long as two years after the treatment.

Means (106) reported that Chapman had increased this series of patients to a total of 65 receiving I^{131} . Forty-six of these had become euthyroid, 11 had become hypothyroid and 8 were improved but still thyrotoxic. No recurrences were seen. Means reported that Chapman had also treated 60 patients with I^{131} . Thirty-six had made satisfactory response, with 3 of this group receiving a second dose of I^{131} ; 12 were better but still somewhat thyrotoxic six months after treatment; the remainder had not been followed long enough to evaluate.

Soley and Miller (137) treated 33 patients who had diffuse toxic goiter by giving monthly doses of 1,000 to 2,000 microcuries of I^{131} until a therapeutic response occurred. Because of its longer half-life, smaller doses may be given than with I^{130} . Twenty patients responded well in from one and one-half to seven months after the treatment had been started, the average being 3.6 months. As a result of treatment the average estimated weight of the thyroid was reduced from 31 to 13 Gm., the basal metabolic rate from plus 28 per cent to minus 10 per cent, and the serum-protein-bound iodine from 10.7 to 5.7 micrograms per 100 cc. of serum. The average dose of radioiodine in the cases in which treatment was successful was 2,726 microcuries with a range from 800 to 4,500 microcuries. The patients whose treatment was unsuccessful had larger glands, higher metabolic rates and higher serum-protein-bound iodine than did the patients whose treatment was successful. Within twenty-four to seventy-two hours after administration of the dose, often there was definite tenderness of the thyroid gland, increased blood sedimentation rate and serum-protein-bound iodine. At four to ten days there was an increase of thyrotoxicity. Failure of these changes to appear often foretold a poor result. Six of twenty-six patients experienced increase of exophthalmos after treatment.

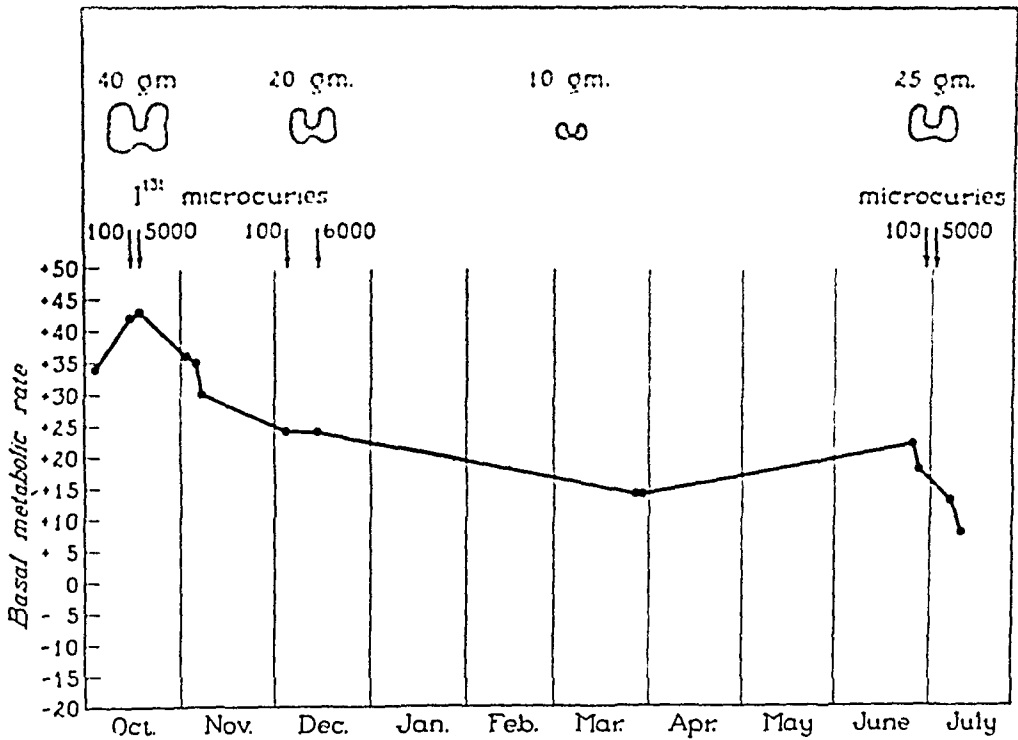
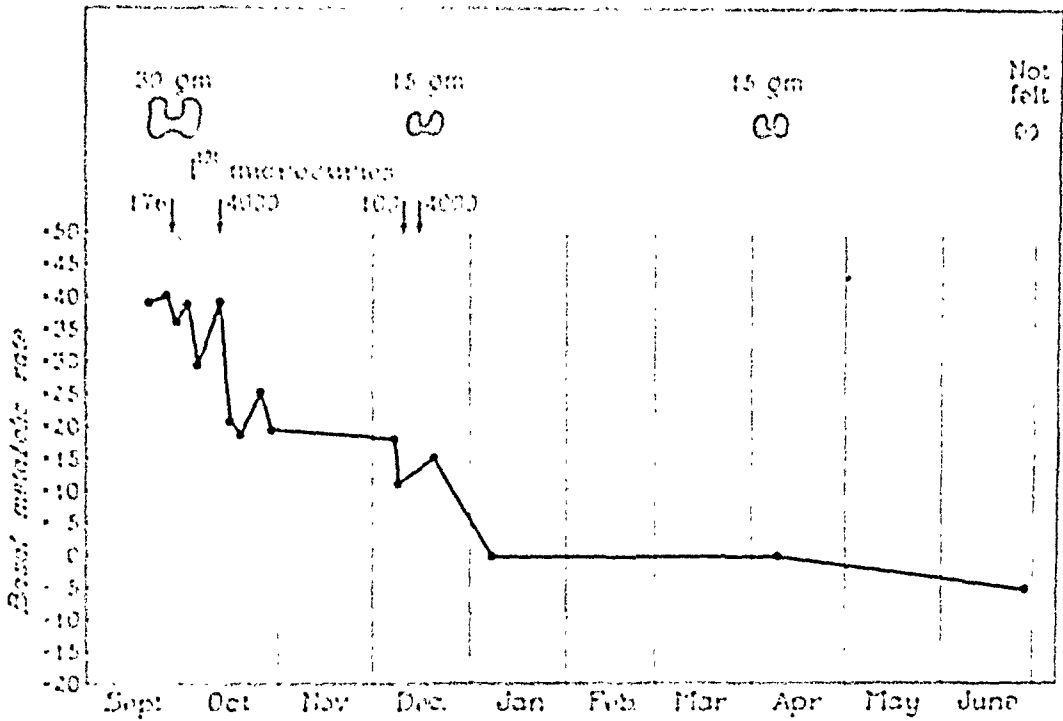
Haines and co-workers (128) calculated the desired dose of I^{131} before administering it. Capacity of the diseased thyroid to collect radioiodine was determined by a preliminary tracer dose. In the realization that most

gen rays or radium, the dosage of which is limited by radiation damage to tissues other than the thyroid, particularly the skin. Radium or roentgen therapy can be given to a total of 6,000 roentgens (124) while as much as 25,000 equivalent roentgens can be safely administered to the thyroid in a single dose of radioiodine for the treatment of hyperthyroidism (128). Much larger doses have been given in cases of thyroidal cancer without demonstrable serious effect (124, 129). On the other hand, incidental damage to ovaries, kidneys or other organs, an increase in the probability of mutations in offspring (130), or the possibility of a carcinogenic effect of radioiodine have not yet been determined (131). In order to calculate the dosage of radioiodine, the energy and type of radiations, the amount collected per gram of tissue and the approximate weight of the tissue must be known. Several papers have appeared which give information on dosage determinations for radioiodine (40, 79, 129, 132, 133). The most important uses of radioiodine are for treatment of hyperthyroidism and carcinoma of the thyroid but it has been used recently to treat euthyroid cardiac patients by producing myxedema (134).

Hyperthyroidism.—The first thyrotoxic patients to receive radioactive iodine as a therapeutic measure were treated by Hertz and Roberts (102) in 1941. Coincidentally, Hamilton and co-workers (12, 135) gave large doses of I^{131} to dogs and rabbits producing marked destruction of functional tissue in the thyroid with replacement by fibrous tissue. Other tissues, including the recurrent laryngeal nerves, the parathyroids and the trachea, remained normal. With this information three patients who had hyperthyroidism were given smaller doses of I^{131} . Within four to six weeks there was marked clinical improvement and in a few months there was complete remission, with one patient requiring a small second dose.

Subsequent studies (136) have confirmed the foregoing brief reports and some patients now have been observed in remission for several years. Hertz and Roberts (104) treated 29 thyrotoxic patients with a mixture of I^{130} , 90 per cent, and I^{131} , 10 per cent, and noted remission in 20 patients. The small dosage of radioiodine (5 to 28 millicuries) and the subsequent administration of ordinary iodine in these cases makes interpretation of results difficult. Chapman and Evans (105) treated 22 patients having exophthalmic goiter with usually larger doses (14 to 79 millicuries) of the same radioiodine mixture. No other treatment was employed. Fourteen of the patients responded to a single dose while 8 required subsequent dosage. Remissions were complete in 20 patients and partial in 2. In 4 patients myxedema developed. Twenty-one of the patients experienced reduction in size of the thyroid to no goiter or a thyroid that was barely palpable. Fibrosis of the thyroid was seen in the two glands which were biopsied. There was a great diminution in the number of follicles; but the epithelium

Eighteen patients had not been followed long enough for evaluation; 76 had been euthyroid from three to twelve months and 4 were myxedematous. Temporary exacerbation of thyrotoxicosis as a result of radiation was controlled in some patients by one of the antithyroid drugs before, and with



of an ingested dose not excreted in the urine within seventy-two hours is collected by the thyroid, this fraction was arbitrarily used as the percentage of an oral dose which the thyroid would collect. Most of the I^{131} collected by the thyroid remains in the gland until its radiations are spent. Weight of the thyroid was estimated in grams after palpation of the patient's neck. Experience proved that in most cases a satisfactory dose of I^{131} delivered to the thyroid was 200 to 250 microcuries per gram of glandular tissue. Dosage was then calculated by the following formula:

$$\frac{\text{Microcuries per gram (200-250)} \times \text{estimated thyroid weight} \times 100}{\text{Per cent of } I^{131} \text{ tracer collected by thyroid}} = \text{desired dosage in microcuries.}$$

The oral doses varied from 2,600 to 20,000 microcuries. In consideration of possible late effects of radiation, therapeutic radioiodine was given to those patients in whom the risk and difficulties of other methods of treatment appeared to be excessive. These patients included older patients having serious heart disease, multiple recurrences of goiter, vocal cord paralysis, a patient with marked iodine sensitivity making preoperative iodination impossible, and one with coexistent malignant disease outside the thyroid. Of the 40 patients treated, 27 had good, 8 had fair, and 5 had poor results after administration of a single dose. In the 8 cases in which response was fair the hyperthyroidism was later controlled by one or two subsequent doses of radioiodine. In the 5 cases in which the results were poor the patients were given inadequate dosage or the hyperthyroidism was complicated by serious heart disease that may have caused wrong diagnoses. In 8 cases myxedema developed but this was a minor complication considering the critical status of some of the patients. Three had recurrence of hyperthyroidism. One instance of brief roentgen-ray sickness and leukopenia occurred (Fig. 13). A striking observation was the unexplainable variability of response of different thyroid glands to approximately the same dosage of radioiodine. The possibility of spotty uptake of radioiodine by exophthalmic goiter may be significant in this phenomenon (10) (Fig. 10a).

Williams (82), in discussing the forms of treatment for hyperthyroidism, listed the following indications for the possible use of radioiodine: 1) inability to get the patient into satisfactory condition for surgical treatment, 2) patient cannot afford surgical treatment, 3) patient fears surgical treatment, 4) multiple recurrences of hyperthyroidism, 5) vocal cord paralysis, 6) extremely large goiter, 7) sensitivity to, refractoriness to, or lack of cooperation in taking antithyroid drugs.

Williams and co-workers (83) treated 98 unselected patients who had thyrotoxicosis with I^{131} . The average dose was 8.5 millicuries given in from one to six doses. Repeat doses were given at intervals of six to eight weeks.

ineffectual therapeutic dose of radioiodine. At necropsy the only metastatic nodule which collected appreciable radioiodine was found to contain colloid follicles while the other metastatic growths, by histologic examination, were undifferentiated. McArthur and Cope (140) gave radioiodine pre-operatively to 18 patients who had discrete thyroid nodules. Six of these nodules turned out to be malignant and collected from none to a fourth as much radioiodine as did adjacent uninvolved tissue. In 3 instances there was detectable collection of radioiodine by metastatic growths.

Five of 12 carcinomas of the thyroid were observed by Rawson and co-workers (118) to collect minimal but measurable amounts of radioiodine. This same degree of function was found to exist in the metastatic growths from 8 of these tumors. Seidlin, Oshry and Yalow (141) demonstrated uptake of radioiodine in the metastatic growths in 8 of 14 thyroidal cancers and treated the positive growths with radioiodine. Soley (120) stated that uptake of radioiodine sufficient for therapeutic purposes was present in the metastatic growths in only 1 of 10 cases of thyroid cancer.

Marinelli, Foote and Hooker (38) studied radioiodine uptake in 19 cases of thyroidal cancer by autoradiography and found 10 neoplasms which took up appreciable amounts of radioiodine; 5 were "benign metastasizing struma" and 5 were follicular adenocarcinoma. Two papillary adenocarcinomas collected only questionable amounts. Considering the incidence of these types of cancer in comparison with the incidence of the types which do not collect radioiodine, about 15 per cent of all thyroidal cancer should collect appreciable amounts of radioiodine. In general only the tumors containing colloid collect radioiodine but certain highly malignant tumors appear to be exceptions to this rule. Not all of the tumors with colloid or follicle formation collected radioiodine. A lack of uniformity of uptake within a cancer was noted. This was not entirely correlated with histologic appearance, for there was no uptake of isotope in some regions the structure of which would ordinarily indicate ability to retain radioiodine. A single biopsy did not always determine whether radioiodine uptake was sufficient for treatment. One case with papillary adenocarcinoma at biopsy of the thyroid showed poor uptake, but Geiger counts showed appreciable uptake in the metastatic growths. Biopsy of the ribs demonstrated a follicular carcinoma.

The impression that there may be a correlation between colloid formation and capacity to collect radioiodine is found in the work of Gorbman (142-146) on the fetal thyroid of rats, which demonstrated that fetal thyroid cannot concentrate iodine until there is differentiation of solid cell cords into follicles and deposition of colloid into the lumina. This transformation occurred at the nineteenth day of fetal life. Chapman (147) has found that human fetal thyroid tissue concentrates radioiodine given to

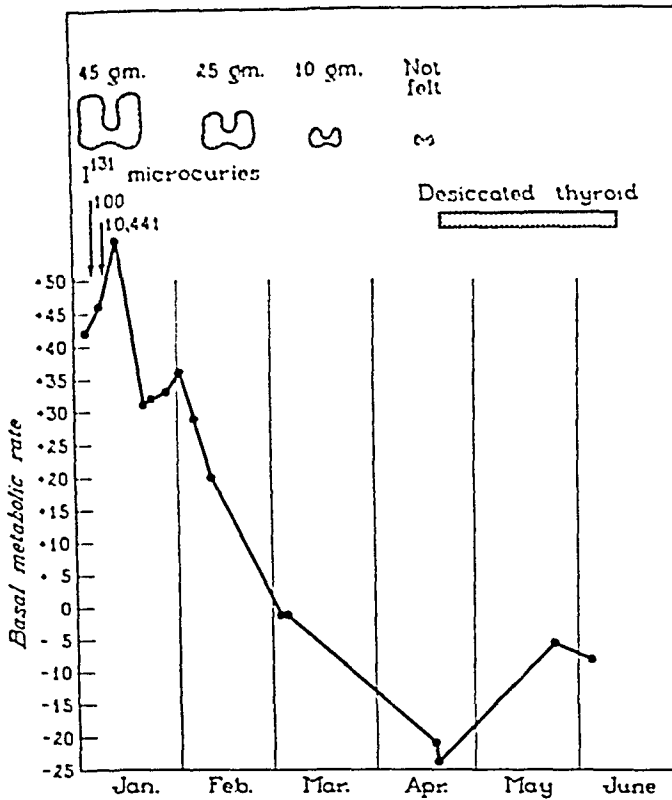


FIG. 13. Sample results in treating exophthalmic goiter with radioiodine. The diagram at the top of each graph represents the estimated size of the thyroid during the course of treatment. The small doses of radioiodine were given for tracer studies, while the larger doses were given for therapeutic purposes. Good result (a), recurrence of hyperthyroidism (b), and occurrence of myxedema (c) are illustrated.

iodide after, the radioiodine was administered. This procedure eventually became routine for all patients. Maximal responses to treatment were noted at six weeks or longer. Soley (120) has stated his conviction "that I^{131} will soon be an accepted satisfactory method of treating a larger proportion of patients with Graves' disease than would be chosen for roentgen therapy and may even replace surgery for the average patient." Haines and co-workers (128) drew no definite conclusions but were interested in its uses for patients who were difficult to control or who were poor surgical risks.

Carcinoma of the thyroid.—Certain types of thyroidal cancer have the capacity of concentrating sufficient quantities of iodine to make internal radiation of them with radioiodine a feasible treatment. Early reports (12, 27) were discouraging but Keston and co-workers (138, 139) soon reported a case in which Geiger counter studies demonstrated the ability of metastatic thyroidal cancer to collect radioiodine. This patient was given an

There is enough tellurium in some doses of radioiodine to produce a physical sign of tellurium ingestion—garlic odor—which may persist for months on the breath of patients treated with large doses of radioiodine for thyroidal cancer (68). Not enough tellurium is ingested to cause toxic symptoms, which would be similar to those of arsenic poisoning.

Addendum. Since this paper was prepared for publication the following articles have appeared, which should be included in this review:

Nickson (Nickson, James J.: Dosimetric and protective considerations for radioactive iodine, *J. Clin. Endocrinol.* 8: 707-717 [Sept.] 1948) gives practical abbreviated formulas for calculating therapeutic dosages of, and exposure of personnel to, radioiodine.

Werner and associates (Werner, S. C.; Quimby, Edith H. and Schmidt, Charlotte: The clinical use of radioactive iodine, *Bull. New York Acad. Med.* 24: 549-560 [Sept.] 1948) reported the treatment with I^{131} of 40 patients who had diffuse toxic goiter. Follow-up studies lasted from four months to more than a year. The dose utilized was determined by measurements over the thyroid with a Geiger counter. There were no failures with a single dose retained by the thyroid of 75 or more microcuries per estimated gram of tissue. Seven of the patients retaining less radioiodine required a second dose. In 4 cases temporary hypothyroidism developed, but this cleared spontaneously.

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the mother only after the fourteenth week of pregnancy or after the fetal thyroid has developed to the point of follicle formation and deposition of colloid.

Only 1 case in which thyroidal cancer was treated with radioiodine has been reported in detail (124, 148). The patient had undergone total thyroidectomy twenty years before treatment with radioiodine and functioning metastatic growths and hyperthyroidism developed five years before. Therapeutic doses totaling 110.8 millicuries of I^{130} and 158 millicuries of I^{131} were given orally over a two-year period, nearly 40,000 equivalent roentgens being delivered to the tumors. During two years of observation there was clinical improvement and arrest of the growth of metastatic tissue but the metastatic tissue still retained the ability to collect radioiodine, indicating that the malignant lesion had not been destroyed. There were no serious reactions to the large doses of radioiodine, even though temporary alopecia occurred over a large metastatic area in the skull, and transient leukopenia developed. Leiter and co-workers (148) quoted Hare, who found no response by an alveolar carcinoma of the thyroid after exposure to 6,000 roentgens of x-radiation, but who successfully treated a patient by interstitial implantation of radon with a calculated dose of 20,000 roentgens, a value well beyond the reach of external radiation.

The maximal dose of radioiodine which a human being can tolerate without serious after-effects has not yet been determined. Gorbman (149) gave young mice huge doses of I^{131} (3 to 50 millicuries per kilogram of body weight). The larger doses produced complete destruction of the thyroid within a few days. The smaller doses permitted survival of some thyroidal epithelium but resulted in loss of the parathyroids. Lesions were produced in the tracheal epithelium with all dosages and in the recurrent laryngeal nerve with the larger doses. Possibly the central regions of the thyroid or of a metastatic growth are exposed to more radiation than the peripheral regions (150). Because of spotty uptake and limited penetration of beta particles, it would be well to deliver large enough doses of radioiodine to get therapeutic gamma irradiation (38). Some patients at the Memorial Hospital in New York have received single doses of 250 millicuries of I^{131} without serious toxic effect (129). Uptake of radioiodine by cancers of the thyroid may be increased by removal of the normal thyroid by surgical means or by internal radiation, by giving antithyroid drugs and withdrawing the drug a few days before administration of radioiodine, by injections of thyrotropic hormone (151) or by temporary renal block of radioiodine excretion (38). Rawson (152) noted that occasionally, as one metastatic nodule is destroyed by radioiodine, another begins collecting the isotope and can be treated. There is a hopeful possibility that radioactive organic compounds may be produced which localize in tumor tissue (153).

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The 1949 Meeting of the American Goiter Association

The next annual meeting of the American Goiter Association will be held at the Loraine Hotel in Madison, Wisconsin, May 26, 27 and 28, 1949.

All members of the Association are urged to make their hotel reservations as early as possible. Those who would prefer a resort hotel will find the new Edgewater very beautiful. It is half a mile from the Loraine on the shores of Lake Mendota.

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Announcement of Jefferson Medical College and Hospital Fellowship

A Fellowship in obstetric and gynecologic endocrinology will be available at the Jefferson Medical College and Hospital, Philadelphia, on or about May 1, 1949, under the direction of Dr. A. E. Rakoff, Assistant Professor of Obstetrics and Gynecology, and Endocrinologist to the Department of Clinical Laboratories.

The Fellowship is available to Doctors of Medicine who have had at least one year or its equivalent of postgraduate training in obstetrics and gynecology. Applicants for the Fellowship should communicate at once with Dr. Lewis C. Scheffey, Professor of Obstetrics and Gynecology, Head of Department, and Director of Division of Gynecology, Jefferson Medical College and Hospital, Philadelphia 7, Pa.

The 1949 Meeting of the Association for the Study of Internal Secretions

The Thirty-First Annual Meeting of the Association for the Study of Internal Secretions will be held in the Chalfonte-Haddon Hall, Friday and Saturday, June 3 and 4, 1949, in Atlantic City, New Jersey.

We are informed by the hotel management that reservations will be difficult to secure on short notice; therefore, members are urged to make reservations at once with Chalfonte-Haddon Hall, giving time of arrival and length of stay in Atlantic City.

The scientific sessions will be held in the Viking Room, as formerly, and registration will be on the same floor. The annual dinner will be held in the Rutland Room, Friday, June 3, at 7 p.m., preceded by cocktails in the same room.

Those wishing to present papers, which will be limited to ten minutes, should send the title and four copies of an abstract of not more than 200 words, to Dr. J. S. L. Browne, Royal Victoria Hospital, Montreal 2, Canada, not later than March 1, 1949. It is imperative that the abstracts be informative and complete, with results and conclusions, in order that they may be of value for reference and suitable for printing in the program.

Nominations for the Squibb and Ciba Awards and the Ayerst, McKenna and Harrison Fellowship should be filed on special forms with the Secretary of the Association, not later than March 15, 1949, according to specifications given in the section on Awards.

The 1949 Annual Meeting of the American Diabetes Association

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For both journals the field of endocrinology is interpreted in its broadest sense. However, in papers from other fields of medicine and biology, the endocrine aspects of the topic must be explicit and significant.

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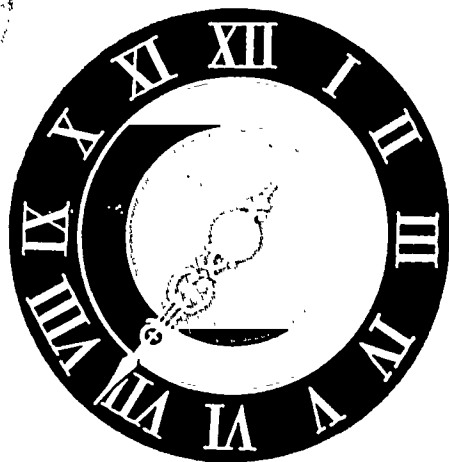
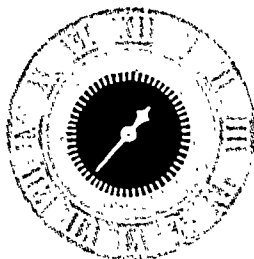
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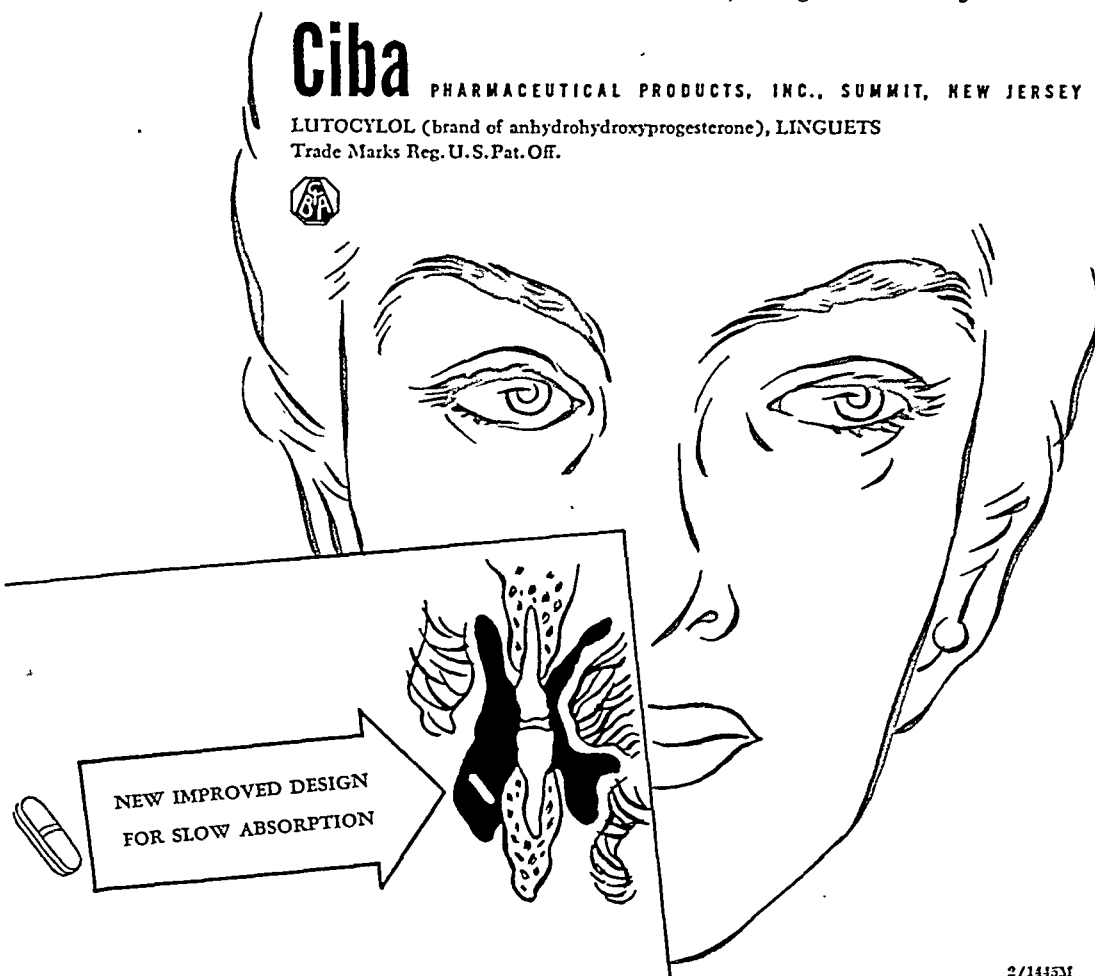
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tract. Administration of this serum to male or female rats was followed by atrophy of the gonads, similar to that occurring after hypophysectomy.

The problem of antihormone formation is therefore of considerable importance in clinical therapy with gonadotropic hormones. Besides vitiating the effects of the administered hormone, the formation of nonspecific antihormones may also nullify the actions of the patient's endogenous gonadotropic hormones. This possibility has been recognized, but as yet has not been demonstrated (3).

Antihormone formation in human subjects has followed administration of pregnant mare serum gonadotropin (4, 5, 6), horse pituitary gonadotropin (7), and a mixture of human chorionic gonadotropin and sheep pituitary gonadotropin ("Synapoidin") (6). The antihormones formed to pregnant mare serum were specific (4, 6), *i.e.*, they were effective only against pregnant mare serum gonadotropin. The antihormones formed to "Synapoidin" and to horse pituitary gonadotropin were nonspecific, as they were effective against pregnant mare serum as well as against the administered hormones (6, 7). There was no evidence that the antihormones were effective against the patients' endogenous gonadotropic hormones (6).

It is the purpose of this communication to report the results of studying antigonadotropin formation in 7 sterile men receiving a sheep anterior pituitary extract rich in follicle-stimulating hormone¹ (hereafter referred to as sheep FSH). The following problems were investigated:

1. Rate, incidence and amount of antihormone formation.
2. Specificity of the antihormones.
3. Effects of the antihormones on the patients' endogenous gonadotropins.
4. Mechanism of action of the antihormones.

Method of antihormone assay: Twenty-four-day old female Sprague-Dawley rats were used as assay animals. A sample assay is presented in Table 1. Each rat received a total of 4 units² of sheep FSH dissolved in 6.0 cc. of water, 1.0 cc. being injected subcutaneously twice daily for three days. One group of rats received only FSH, whereas others received in addition varying amounts of plasma suspected of containing antihormones (obtained from patient K.H. eighty-two days after initiating therapy). The plasma was divided into 6 equal doses and injected subcutaneously concurrently with the hormone, but at a separate site. The rats were killed

¹ Generously supplied by the Schering Corporation through the courtesy of Dr. Edward S. Henderson. (This preparation also contained small amounts of interstitial cell-stimulating hormone.)

² "Units" refers to the manufacturers' stated potency. This also applies to the other hormones used.

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ANTI-HORMONE FORMATION COMPLICATING PITUITARY GONADOTROPIN THERAPY IN INFERTILE MEN

I. PROPERTIES OF THE ANTIHORMONES*

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PROLONGED administration of gonadotropic hormones derived from one species to an individual of a different species can elicit formation of substances in the plasma capable of preventing the action of the administered hormones. These substances have been named *antihormones*, or more specifically, *antigonadotropins*. Such terminology infers a similarity between antihormone and antibody formation; however, it is not the purpose of this report to consider the immunological properties of antihormones. This subject has been amply reviewed by Zondek and Sulman (1).

Antigonadotropins, besides being effective against the administered hormone, may also be effective against other gonadotropic hormones. In experimental animals, it has been demonstrated that nonspecific antigonadotropins may even be effective against the normal, circulating gonadotropins. For example, Rowlands (2) found that nonspecific antihormones were formed in the serum of rabbits injected with an ox anterior pituitary ex-

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no detectable difference, as both are at uninjected control levels. However, uterine weights now show a clear-cut difference, 146 *vs.* 37 mg.

The individual assay results for each patient are tabulated in Tables A to G in the appendix, and summarized in Table 2.

TABLE 2. RATE AND AMOUNT OF ANTIGONADOTROPIN FORMATION TO SHEEP FSH

Patient	Detection of anti-hormones before therapy	Duration of therapy (days) (50 units sheep FSH daily)	Anti-hormones first detected (days)	Maximal amount (units of anti-hormone* in circulation)	Last day found present (after stopping therapy)	First day found absent (after stopping therapy)
D.B.	None	65	52	3,000	37	86
H.B.	None	104	51	5,000	39	88
R.C.	None	84	60	>7,000	60	125
L.D.	None	82	49	10,000	58	108
C.G.	—	60	56	>7,000	283	—
K.H.	None	66	52	10,000	128	164
G.M.	—	56	45	3,000	21	121
Range	None (5 tested)	56-104	45-60	3,000-10,000	21-283	86-

* One "antihormone unit" is that amount just sufficient to prevent the action of 1 unit of sheep FSH.

RATE, INCIDENCE AND AMOUNT OF ANTIHORMONE FORMATION

Antihormone titers before therapy: Antihormone titers were determined in 5 of the 7 patients before initiating therapy by assaying 0.9 cc. of plasma against 2 units of sheep FSH. In 2 normal students 6.0 cc. of plasma was assayed against 4 units of FSH (Table H, appendix). In no instance were antihormones detected in untreated individuals.

Duration and amount of therapy: Each patient received 50 units of sheep FSH daily (self-administered, intramuscularly) except patients K.H. and G.M. Patient K.H. received 50 units twice daily for twelve days and then 50 units once daily for fifty-four days. Patient G.M. received 50 units twice daily for forty-five days and then 50 units once daily for eleven days. Duration of therapy ranged from 56 to 104 days, and is indicated for each patient in Table 2. Pretreatment intradermal sensitivity tests to the sheep FSH were negative in each patient.

Incidence of antihormone formation: Antihormone formation occurred in each of the 7 patients. By determining antihormone titers at approximately two to four week intervals it was found that all 7 patients developed antihormones within forty-five to sixty days after initiating therapy (Table

twenty-four hours after the last injection, and the weights of ovaries and fluid-free uteri were taken as the assay end points. (Controls receiving only FSH were included in each assay, because it was found that the amount of hormone varied from ampule to ampule. It should be stressed, however, that in each assay all rats received exactly the same amount of hormone from the same stock solution, freshly prepared prior to each assay.)

The data of Table 1 demonstrate that rats receiving only FSH showed marked uterine and ovarian stimulation. Rats receiving in addition 0.9 cc. of plasma showed only uterine stimulation, whereas rats receiving 1.8 cc. of plasma showed neither ovarian nor uterine stimulation.

TABLE 1. DETECTION OF ANTIGONADOTROPINS

Units of sheep FSH per rat*	Volume of plasma in- jected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
4	0	109	83	3
4	0.9†	146	15	2
4	1.8‡	37	13	3
Uninjected controls‡	0	36	13	63

* The FSH and plasma were divided into 6 equal doses administered twice daily for 3 days.

† Source of plasma: K.H., 82 days following initial FSH injection.

‡ These values for uninjected controls were obtained from controls included in each assay and apply to all the data.

Certain details concerning the response of the immature female rat to gonadotropic hormones should be emphasized. The first response of the ovary to gonadotropic hormones is secretion of estrogen, which is detected by an increase in weight of the uterus. Only after the uterine weight increase has reached a maximum, does the ovary increase in weight (8). The importance of this concept is illustrated by inspection of the data in Table 1. Compare first the data for rats receiving only FSH with those for rats receiving in addition 0.9 cc. of plasma. If only uterine weight is considered, no difference between the two groups can be detected, because both 109 and 146 mg. can be considered as approximately maximal uterine weights (8). However, a clear-cut difference is found by comparing ovarian weights, 83 *vs.* 15 mg. Similarly, compare the figures for rats receiving 0.9 cc. of plasma with those of rats receiving 1.8 cc. of plasma. Ovarian weights show

TABLE 3. ANTIGONADOTROPIC EFFECT ON HUMAN CHORIONIC GONADOTROPIN

Units of chorionic gonadotropin per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
"Pranturon" (Schering)				
1	0	91	22	3
1	1.8*	29	9	3
"A.P.L." (Ayerst)				
1	0	125	19	3
1	3.0†	45	13	3

Source of plasma: * L.D., 105 days following initial FSH injection.

† C.G., 264 days following initial FSH injection.

Antigonadotropic effect of plasma on pregnant mare serum gonadotropin: Three cc. of patient C.G.'s plasma completely prevented the action of 5 units of pregnant mare serum ("Antex," Ayerst, McKenna & Harrison) (Table 4).

TABLE 4. ANTIGONADOTROPIC EFFECT ON PREGNANT MARE SERUM (PMS)

Units of PMS ("Antex," Ayerst) per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
5	0	135	15	3
5	3.0*	41	15	3

* Source of plasma: C.G., 265 days following initial FSH injection.

Antigonadotropic effect of plasma on horse FSH: The antihormones failed to alter the effect of 1 unit of horse pituitary gonadotropin ("Gonatropo," Forbes). Although 1.8 cc. of patient D.B.'s plasma collected sixty-six days after initiating FSH therapy was capable of preventing the response of about 2 units of sheep FSH, the same amount of this plasma failed to affect the response of 1 unit of horse pituitary gonadotropin. Similarly, 3.0 cc. of patient C.G.'s plasma collected 264 days after initiating therapy prevented the effect of approximately 2 units of sheep FSH; but 3.0 and 6.0 cc. of this plasma were without effect on 1 unit of the horse pituitary

2). At this time, antihormone titers were sufficiently great to nullify many times the administered daily dose of sheep FSH.

Amounts of antihormone present in the circulation: The amounts of antihormone present in the circulation can be roughly estimated as follows: from Table 1, it can be seen that 0.9 cc. of K.H.'s plasma almost, but not completely, prevented the action of 4 units of FSH. It can be estimated that approximately 3 units of FSH were inactivated. Defining an "antihormone unit" as that amount just sufficient to prevent the action of 1 unit of hormone, then 0.9 cc. of K.H.'s plasma contained about 3 units of antihormone. Assuming total plasma volume as 3,000 cc., then there were $3,000/0.9 \times 3$, or 10,000 units of antihormone in the total plasma. This is enough to neutralize 200 times the administered daily dose, or almost 3 times the total amount of hormone administered. Similar estimations for the other patients showed that at the time of maximal antihormone formation there were 3,000 to 10,000 units of antihormone present in the plasma (Table 2).

Time required for antihormones to disappear: Repeated antihormone assays performed after stopping therapy demonstrated that antihormones disappeared from the plasma within three to five and one-half months after the last injection of FSH in 6 of the 7 patients (Table 2). At the date of the last test, 6.0 cc. of plasma of each of these patients did not alter the effects of 4 units of sheep FSH. Antihormones were still present in the other patient 283 days after cessation of therapy; at this time the titer was approximately one-fourth of the maximal value.

SPECIFICITY OF THE ANTIHORMONES

In order to test the specificity of the antihormones, plasma containing antihormones to sheep FSH was tested against the following gonadotropic hormones:

1. Chorionic gonadotropin derived from human pregnancy urine.
2. Pregnant mare serum gonadotropin.
3. Anterior pituitary gonadotropin derived from horse pituitaries (mainly FSH).
4. Urinary gonadotropin derived from the urine of a castrated man (mainly FSH).

The assays were carried out as previously described.

Antigonadotropic effect of plasma on human chorionic gonadotropin: Two commercial preparations were tested: "Pranturon" (Schering) and "A.P.L." (Ayerst, McKenna & Harrison). In both instances, 1 unit of hormone elicited a definite gonadotropic response which was completely prevented by the injection of 1.8 and 3.0 cc. of plasma of patients L.D. and C.G., respectively (Table 3).

EFFECTS OF THE ANTIHORMONES ON THE PATIENTS'
ENDOGENOUS GONADOTROPINS

Since the antihormones were sufficiently nonspecific to be active against human castrate male gonadotropins, the question arose whether or not they were effective against the patients' own urinary gonadotropins. Antihormones were tested against endogenous gonadotropins in 4 of the patients. Urine collected after stopping therapy (in order to avoid recovering

TABLE 7. EFFECT OF ANTIHORMONES ON PATIENTS' ENDOGENOUS GONADOTROPINS

Amount ultrafilter urine concentrate per rat	Total cc. plasma per rat	Days after therapy initiated, plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
12-hour urine per rat collected by R.C., days 54 to 63 after stopping therapy	0	—	86	12	3
	6.0	144	33	12	3
12-hour urine per rat collected by L.D., days 2 to 7 after stop- ping therapy	0	—	173	22	3
	3.0	91	28	12	3
12-hour urine per rat collected by K.H., days 6 to 9 after stop- ping therapy	0	—	113	12	2
	6.0	95	26	11	2
12-hour urine per rat collected by C.G., days 4 to 7 after stop- ping therapy	0	—	135	16	2
	0.9	77	28	11	2
4-hour urine per rat collected by C.G., days 126 to 131 after stopping therapy	0	—	82	14	4
	6.0	193	33	14	2

administered sheep FSH) was concentrated by ultrafiltration. Each rat in a given assay received an equal aliquot of the ultrafiltered urine concentrate. One group received only urine concentrate, whereas others received, in addition, plasma from the same patient. For example, patient L.D. collected urine from day 2 to day 7 after stopping therapy. The hormone concentrated from a twelve-hour urine specimen elicited definite uterine and ovarian stimulation; this was completely prevented by the addition of 3.0 cc. of his own plasma (Table 7). Similar results were obtained on the other 3 patients (Table 7).

preparation (Table 5). Thus, plasma containing antihormones formed in response to sheep FSH was not antigonadotropic to horse pituitary gonadotropin.

TABLE 5. ANTIGONADOTROPIC EFFECT ON HORSE ANTERIOR PITUITARY EXTRACT

Units of horse FSH ("Gonatropin," Forbes) per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
1	0	150	20	9
1	1.8*	120	18	3
1	3.0†	116	20	3
1	6.0†	138	24	3

Source of plasma: * D.B., 66 days following initial FSH injection.

† C.G., 264 days following initial FSH injection.

Urinary gonadotropin obtained from a castrated man: The urine was concentrated by the ultrafiltration technic (9). A two-hour aliquot gave a definite gonadotropic response which was partially prevented by 0.9 cc. of plasma and completely prevented by 6.0 cc. of plasma (collected from patient K.H. sixty-seven days after initiating therapy) (Table 6).

TABLE 6. ANTIGONADOTROPIC EFFECT ON HUMAN URINARY GONADOTROPIN

Aliquot hours; human male castrate ultra- filter urine concentrate per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
2	0	114	55	3
2	0.9*	117	32	2
2	6.0*	21	5	3

* Source of plasma: K.H., 67 days following initial FSH injection.

Antihormones that form in response to administration of sheep FSH are effective against hormones from sheep, horse, and human sources, and therefore *are not species specific*. The antihormones are effective against anterior pituitary, human chorionic, and pregnant mare serum hormones, and therefore *are not hormone specific*. However, this lack of specificity is not complete, because the antihormones are not effective against horse pituitary gonadotropin.

and Wolfe (10), referring to the unpublished data of Meyer and Sevringhaus, stated that antigonadotropins were formed in the blood of human females after the administration of anterior pituitary gonadotropin preparations; no further information was given. Spence, Scowen and Rowlands (11) were unable to detect antihormones in the serum of 2 patients following treatment with 30 to 50 units of a pig pituitary extract twice weekly for sixteen to twenty-three weeks. Leathem and Rakoff (7) reported that 6 of 13 patients treated with 200 to 400 units of horse pituitary gonadotropin per month developed antihormones after three to four months of therapy.

To our knowledge there are no previous data concerning the formation

TABLE 8. RECOVERY OF ANTIHORMONES FROM PLASMA BY ULTRAFILTRATION

Units sheep FSH per rat	Total cc. plasma per rat	Treatment of plasma	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
4	0	—	120	52	3
4	1.8*	untreated	97	15	3
4	3.0*	untreated	28	12	3
4	3.0*	ultrafiltration	80	17	3
4	0	—	101	77	3
4	0.9†	untreated	118	31	2
4	1.8†	ultrafiltration	118	29	3

Source of plasma: * C.G., 243 days following initial FSH injection.

† K.H., 109 days following initial FSH injection.

of antigonadotropins in the human following the administration of only sheep pituitary extracts. However, Rakoff and Leathem (12) reported the results of treating 25 patients with a mixture of sheep pituitary and human chorionic gonadotropins ("Synapoidin"). Antihormones did not form in 22 patients treated for two to five months. Antihormones did form in 3 patients treated for more than six months with this preparation. The formation of antihormones in their experiments is most probably due to the sheep FSH in the extracts, since antihormone formation has not been demonstrated following treatment with human chorionic gonadotropin alone. (Segaloff and Parson (13) have reported the presence of antihormones effective against human chorionic gonadotropin after the administration of this material. However, as antihormone assays were not performed prior to treatment with chorionic gonadotropin, and as this patient had previously received protracted treatment with a pituitary extract, it is most

The following control experiments were also performed: 6.0 cc. samples of plasma from normal medical students R.B., L.C., and E.J. were tested against twelve-hour urine extracts from patients C.G., K.H. and L.D. respectively, without materially effecting the gonadotropic response of the extracts (Table H, appendix). Also, the plasma of normal subject E.J. was not antigonadotropic to his own urine extracts (Table H, appendix).

MECHANISM OF ACTION OF THE ANTIHORMONES

Although the antihormones are effective against endogenous gonadotropic hormones, gonadotropins are being excreted in the urine at a time when the antihormones in the blood are at their highest level (compare the time of urine collections (Table 7) with antihormone assays tabulated in the appendix). Also, urinary gonadotropin titers were as high or higher than pretreatment levels.¹ Therefore, antihormones do not destroy or irreversibly combine with gonadotropins nor do they prevent production or release of gonadotropic hormones from the hypophysis. As gonadotropic potency is clearly demonstrable in the urine extracts when antihormones are present in the plasma, separation of antihormone and hormone must occur. There are two possible sites where this could take place:

1. The kidney. The kidney could retain antihormone, but excrete hormone.
2. The ultrafilter concentration procedure. It is possible that the kidney excretes both hormone and antihormone and that ultrafiltration recovers *only* hormone from the urine.

To differentiate between these two possibilities, plasma known to contain antihormones was diluted 1:100 with water and then concentrated by the usual ultrafiltration technic. The concentrate was then extracted with water to the original volume of plasma and its antihormone content compared with that of the original plasma by assaying against sheep FSH. The data of Table 8 show that the ultrafiltration technic is capable of retaining antihormones, and that approximately 50 per cent of the original potency is recovered. Thus, the second possibility is not in operation, and separation of antihormones and hormones must take place at the kidney.

DISCUSSION

Rate and incidence of antihormone formation: Clinical data concerning the formation of antigonadotropins to pituitary extracts are meager. Meyer

¹ See Table I in part II of this study (Jungck, E. C.; Maddock, W. O.; Heller, C. G., and Nelson W. O.) to follow in the April issue of THE JOURNAL OF CLINICAL ENDOCRINOLOGY.

of hormone and antihormone with N/10 to N/15 NaOH and found that the antihormone was destroyed, leaving active gonadotropin.

Kupperman, Meyer and Hertz (21, 22) have concluded from experiments in parabiotic rats that antihormones do not act on the pituitary gland, but that they "neutralize" gonadotropins in the blood stream. We would agree with their conclusions and define "neutralization" as the formation of a combination of gonadotropin and antihormone.

That antihormones are not excreted in the urine of men with high plasma antihormone titers agrees with work in experimental animals (23, 24),

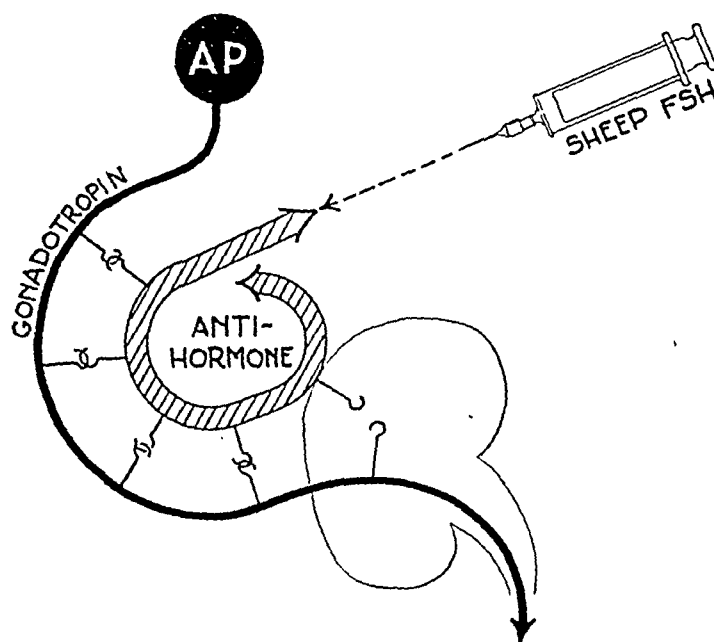


FIG. 1

where antihormones were not detected in the urine of animals having high titers of antihormones in the plasma.

The relationships between antihormones and endogenous gonadotropins are summarized diagrammatically in Figure 1. Administration of sheep FSH elicits the formation of nonspecific antihormones that are effective against the patients' endogenous gonadotropins. Antihormones act by combining with gonadotropins in the blood stream. The antihormone-hormone combination is separated by the kidney, antihormone being retained in the circulation and hormone being excreted in the urine.

SUMMARY AND CONCLUSIONS

1. In each of 7 sterile men given daily injections of follicle-stimulating hormone (FSH) derived from sheep anterior pituitary glands, antihormone formation occurred.

likely that these antihormones were formed in response to the pituitary extract—not the chorionic gonadotropin.)

The low incidence of antihormone formation encountered by Rakoff and Leatham (12) compared to the 100 per cent incidence found by us may be explained on the basis of the difference in dosage employed. Whereas we employed 50 units of sheep FSH daily, they used only 90 units of "Synapoidin" monthly ("15 rat synergy units . . . 3 times weekly for the first 2 weeks of each cycle").

The lack of specificity of the antihormones formed in response to sheep FSH is in agreement with animal experiments (14, 15). The fact that the antihormones were not effective against horse pituitary gonadotropin is puzzling, but not without precedent. Simmonet and Michel (16) found that antihormones formed in rabbits following administration of human chorionic, pregnant mare serum, and postmenopausal urine gonadotropins were not effective against horse pituitary extracts. The only antihormone effective against horse pituitary gonadotropin was that formed in response to horse pituitary extracts.

Our data demonstrate that antihormones do not destroy, irreversibly combine with or prevent pituitary production or release of gonadotropins. Two alternate mechanisms of action may be considered:

1. The antihormones have no effect on gonadotropins, but act directly on the gonads, rendering them incapable of responding to gonadotropins.
2. Antihormone and hormone combine, in which form the hormone is incapable of acting on the gonads. Such a combination could, by definition, be separated by the kidney.

The first mechanism of action seems highly improbable. It is difficult to visualize how an antihormone could render the gonads incapable of responding to one hormone but not another, unless the mechanism of gonadotropic action varies as to the hormone employed. Okkels (17) has shown that thyroid tissue, unresponsive to the action of thyrotropin, again becomes responsive to the same thyrotropin when the thyroid gland is removed and placed in a perfusion apparatus. Selye, Collip and Thomson (18, 19) have demonstrated that rats refractory to one gonadotropic hormone are still capable of responding to other gonadotropins.

The second mechanism of action adequately explains the data. Whether or not an antihormone was effective against a given hormone would depend upon the ability of the two to combine. Such a theory explains the action of antihormones without assuming that they destroy hormones or act directly on the gonads.

Zondek and Sulman (20) demonstrated that antigonadotropins do not destroy gonadotropins *in vitro*. They treated a mixture of equal amounts

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2. Sufficient antihormones formed within two months to vitiate completely the effects of the injected FSH.

3. The maximal titer of antihormones, attained within three months after initiating therapy, was sufficient to neutralize the effect of from 50 to 200 times the daily injected dose of FSH.

4. Antihormones disappeared from the circulation within three to nine months after stopping therapy.

5. Inasmuch as the antihormones were effective against gonadotropins derived from sheep, horse and human sources, they are not species specific; and inasmuch as they were effective against anterior pituitary, chorionic and pregnant mare serum gonadotropins, they are not hormone specific.

6. The antihormones found in the patients' plasma were capable not only of inactivating urinary gonadotropins derived from a castrated man, but also were able to prevent the action of the urinary gonadotropins of their hosts. It is therefore concluded that antihormones can prevent the actions of endogenous gonadotropins.

7. The mechanism whereby antihormones prevent the actions of gonadotropic hormones is apparently a reversible combination between the molecules of hormone and the molecules of antihormone.

It seems unlikely that antigonadotropins destroy gonadotropins or irreversibly combine with them, because endogenous gonadotropic hormones are being excreted in the urine when maximal amounts of antihormone are present in the plasma. It is concluded that the kidney separates the combination of hormone and antihormone, permitting the excretion of gonadotropin and simultaneously retaining antigonadotropins in the circulation.

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TABLE B. PATIENT H.B.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 104 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	111	31	3
2	—	—	113	37	3
2	0.9	24	103	41	3
2	—	—	138	66	3
2	0.9	51	130	18	3
4	—	—	109	83	3
4	1.8	80	116	36	3
4	—	—	109	64	3
4	1.8	93	129	15	3
4	—	—	101	77	3
4	1.8	105	127	20	3
4	—	—	120	52	3
4	1.8	143	121	27	3
4	3.0	143	122	17	3
4	—	—	90	67	3
4	6.0	192	111	67	3

APPENDIX

TABLE A. PATIENT D.B.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 65 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	110	16	4
2	0.9	before therapy	100	29	4
2	—	—	51	11	3
2	0.9	24	53	9	3
4	—	—	109	83	3
4	1.8	39	102	70	3
4	—	—	109	64	3
4	1.8	52	124	32	3
4	—	—	101	77	3
4	1.8	66	107	41	3
4	—	—	93	54	3
4	3.0	81	98	33	3
4	—	—	120	52	3
4	3.0	102	111	21	3
4	6.0	102	115	19	3
4	—	—	90	67	3
4	6.0	151	108	56	3

TABLE D. PATIENT L.D.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 82 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	104	28	3
2	—	—	113	37	3
2	0.9	22	107	38	3
2	—	—	134	32	3
2	0.9	36	138	26	3
2	—	—	138	66	3
2	0.9	49	143	12	3
4	—	—	109	83	3
4	1.8	78	143	13	3
4	—	—	109	64	3
4	0.9	91	133	17	2
4	1.8	91	29	12	2
4	—	—	101	77	3
4	0.9	105	130	29	3
4	—	—	120	52	3
4	0.9	141	106	45	3
4	1.8	141	113	26	3
4	—	—	90	67	3
4	1.8	190	97	70	3
4	3.0	190	102	66	3
4	—	—	111	51	3
4	6.0	227	106	53	3

TABLE C. PATIENT R.C.: ANTIHORMONE TITERS TO SHEEP FSH
 Treatment: 50 units sheep FSH daily for 84 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	103	17	3
2	—	—	51	11	3
2	0.9	45	72	12	3
4	—	—	109	83	3
4	1.8	60	146	54	3
4	—	—	109	64	3
4	1.8	73	151	20	3
4	—	—	101	77	3
4	1.8	87	41	12	3
4	—	—	120	62	3
4	0.9	137	124	59	3
4	1.8	137	133	48	3
4	—	—	101	50	3
4	3.0	144	154	39	3
4	—	—	111	51	3
4	6.0	209	149	54	3

TABLE F. PATIENT K.H.: ANTIHORMONE TITERS TO SHEEP FSH
 Treatment: 50 units sheep FSH twice daily for 12 days
 then 50 units once daily for 54 days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	110	16	4
2	0.9	before therapy	113	25	4
2	—	—	113	37	3
2	0.9	27	100	25	3
2	—	—	138	66	3
2	0.9	53	41	11	3
2	—	—	51	11	3
2	0.9	67	23	8	3
4	—	—	109	83	3
4	0.9	82	146	15	2
4	1.8	82	37	13	3
4	—	—	109	64	3
4	0.9	95	118	21	3
4	—	—	101	77	3
4	0.9	109	118	31	2
4	—	—	120	62	4
4	1.8	159	114	30	3
4	—	—	90	67	3
4	1.8	194	99	87	2
4	4.5	194	99	47	3
4	—	—	111	51	3
4	6.0	230	127	47	3

TABLE E. PATIENT C.G.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 60 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	139	39	4
2	0.9	56	33	12	4
2	—	—	106	39	3
2	0.9	77	41	13	4
2	—	—	98	27	5
2	0.9	95	29	12	5
2	—	—	113	37	3
2	0.9	125	54	17	3
2	—	—	134	32	3
2	0.9	137	27	15	3
4	—	—	109	83	3
4	0.9	180	151	40	2
4	1.8	180	44	16	3
4	—	—	109	64	3
4	1.8	193	36	19	3
4	—	—	93	54	3
4	1.8	222	107	23	3
4	—	—	120	52	3
4	1.8	243	97	15	3
4	3.0	243	28	12	3
4	—	—	101	50	3
4	1.8	264	136	44	3
4	3.0	264	93	20	3
4	—	—	102	64	3
4	3.0	343	110	30	3
4	6.0	343	59	19	3

THE PREGNANDIOL PRECIPITATION TEST. FURTHER OBSERVATIONS ON CLINICAL APPLICATIONS AND TECHNIC*

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THE value of the pregnandiol precipitation test as a rapid laboratory aid in the diagnosis of pregnancy and as a means for predicting the probable outcome of threatened abortion has been indicated in previous reports (1, 2, 3, 4). Added experience with the method has since led to certain modifications of technic. An appraisal of its efficiency based on 737 pregnancy tests and a study of 149 patients with threatened abortion is now presented. Further possible applications of the method in investigation of normal and abnormal ovarian cycles are suggested.

Briefly stated, the pregnandiol test developed in this laboratory depends upon the precipitation of unpurified pregnandiol from the urine of women during the postovulatory phases of normal menstrual cycles and during amenorrhea of normal pregnancy. The presence of pregnandiol during the ovarian cycle is indicative of antecedent ovulation; its presence during amenorrhea of normal pregnancy indicates presumably normal corpus luteum and placental function. The physiologic basis for these assumptions has been well established by numerous investigations using other technics, principally the more time-consuming quantitative methods. Our studies, using the greatly simplified qualitative precipitation test, closely parallel the findings of other investigators.

Recent applications of the precipitation method in studies of normal and abnormal menstrual cycles, correlating the excretion of pregnandiol to fluctuations of basal body temperature, have shown a close relationship of pregnandiol excretion to the postovulatory temperature rise. In cycles with typical biphasic temperature patterns, we have consistently observed pregnandiol excretion following presumptive ovulation.

The amounts of pregnandiol precipitate varied considerably. Pregnan-diol excretion occurred most commonly between the second and fifth days following ovulation and disappeared usually one to four days before the onset of the menstrual flow and the fall of morning temperature.

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TABLE G. PATIENT G.M.: ANTIHORMONE TITERS TO SHEEP FSH
 Treatment: 50 units sheep FSH twice daily for 45 days
 then 50 units once daily for 11 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
4	—	—	109	83	3
4	1.8	22	95	74	3
4	—	—	101	77	3
4	1.8	45	115	55	3
4	—	—	120	52	3
4	3.0	77	77	18	3
4	—	—	102	64	3
4	6.0	177	85	65	3

TABLE H. CONTROL DATA, TESTING PLASMA FROM NORMAL MEDICAL STUDENTS R.B.,
 L.C. AND E.J. AGAINST SHEEP FSH AND URINARY GONADOTROPINS

Hormone	Plasma: amount and source	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
4 units sheep FSH	—	120	62	4
	6.0 cc.—L.C.	114	52	3
	6.0 cc.—E. J.	121	52	3
12-hour ultrafilter urine con- centrate, patient C.G., 7 mo. after stopping therapy	—	89	77	3
	6.0 cc.—R.B.	107	86	3
12-hour ultrafilter urine con- centrate, patient K.H., 4 mo. after stopping therapy	—	125	23	3
	6.0 cc.—L.C.	132	14	3
12-hour ultrafilter urine con- centrate, patient L.D., 3 mo. after stopping therapy	—	107	69	3
	6.0 cc.—E. J.	137	71	3
12-hour ultrafilter urine con- centrate, normal medical stu- dent, E.J.	—	105	44	3
	6.0 cc.—E. J.	112	35	3

cycles usually conformed to the "normal" pattern as shown in the April and May curves. During the June cycle, ovulation occurred late in the fourth week (26th day) instead of at the usual time (14th-16th day). Pregnandiol excretion was first observed on the 28th day. Late ovulation

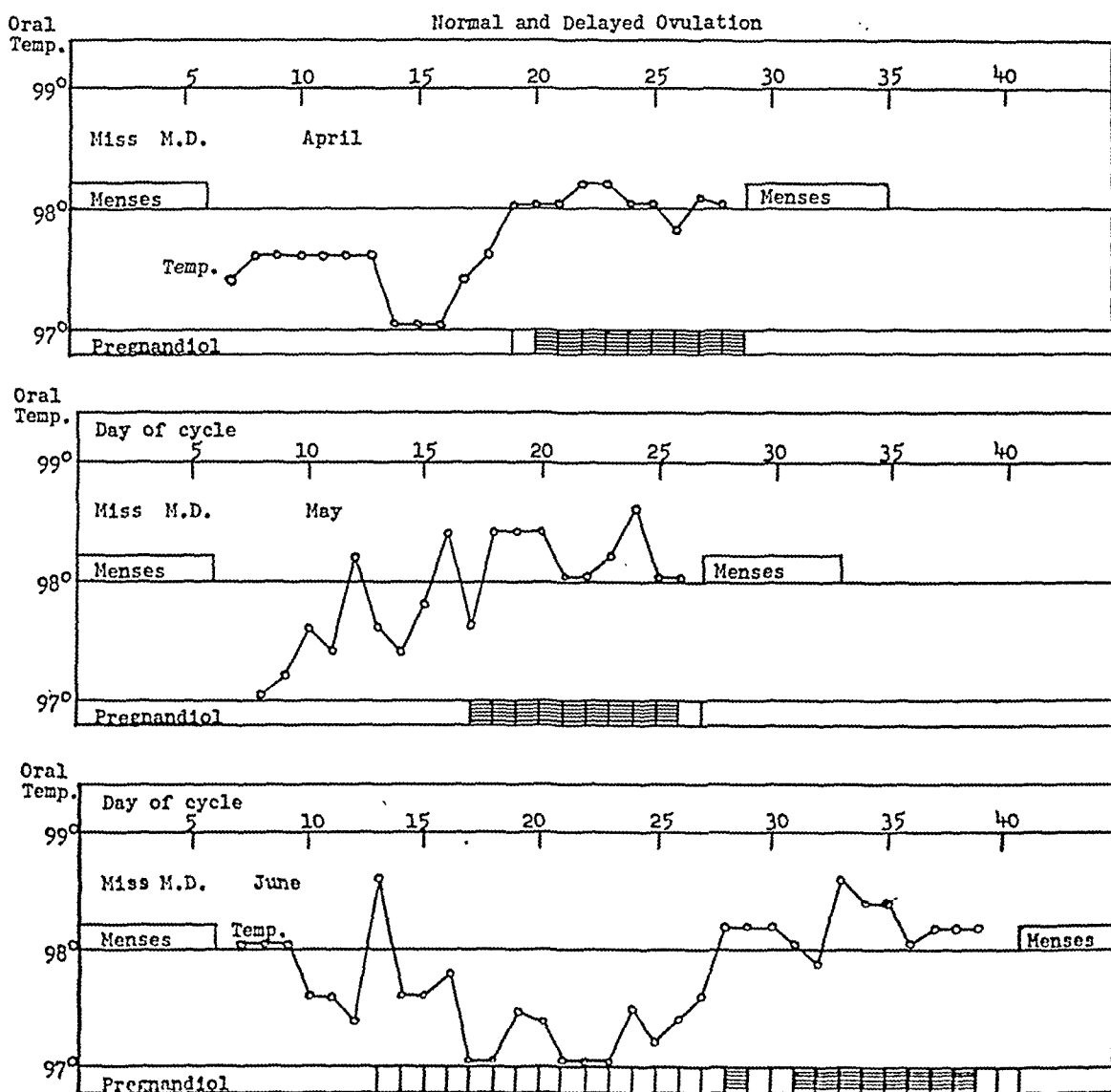


FIG. 3. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

and late pregnandiol excretion were thus followed by delayed menstruation on the 42nd day. Such occurrences explain certain false positive pregnancy tests as will be discussed below.

Figure 4 illustrates data in a patient (Mrs. W. R. T., para 1, gravida 1) under study because of sterility of five years' duration. In March she exhibited a 29-day cycle with complete absence of pregnandiol excretion; in

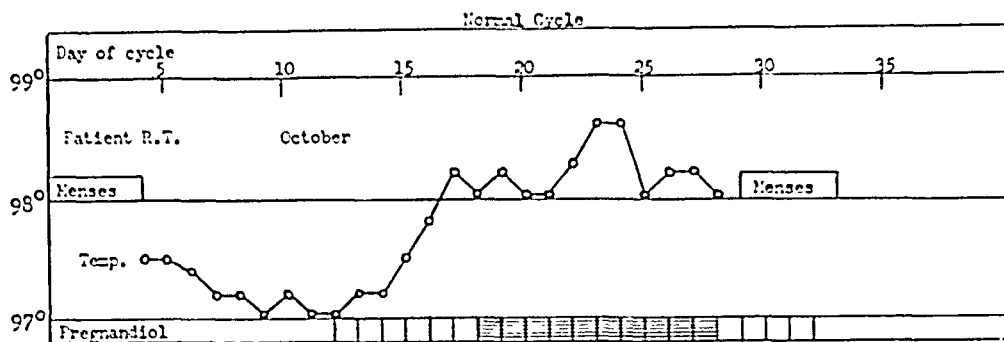


FIG. 1. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

Figure 1 graphically illustrates a typical relationship between basal temperature and pregnandiol excretion in a normal subject, Mrs. R. T., a 24-year-old white married female who became pregnant three months later. The time of appearance of the precipitate following the postovulatory temperature rise and its absence on the day preceding the menses is typical of our findings in other normal, ovulatory cycles.

Figure 2 (Mrs. A. D., para 1, gravida 2) illustrates an instance in which the relationship of pregnandiol excretion to basal body temperature was observed during the cycle preceding the onset of a planned pregnancy. Continued excretion of pregnandiol and the sustained temperature level following ovulation are typical of normal pregnancy.

Figures 1 and 2 illustrate normal cycles of regular interval in which the ovulatory temperature rise and postovulatory pregnandiol excretion occur in typical sequence.

Figure 3 (M. D., para 0, gravida 0) illustrates an example of delayed ovulation and late pregnandiol excretion in a subject whose menstrual

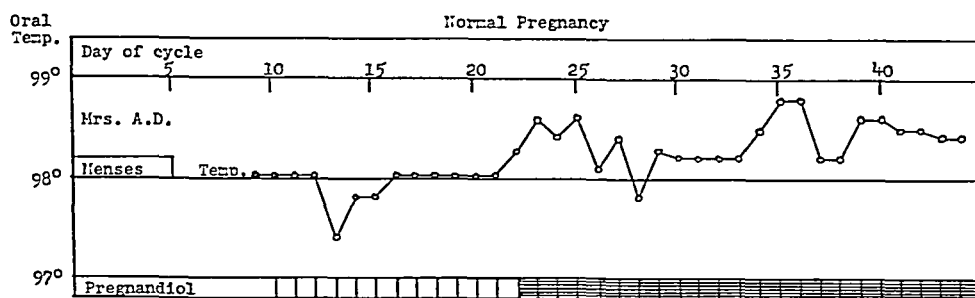


FIG. 2. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

TECHNIC

Experience gained in performing more than 6000 determinations has led to certain valuable modifications of the procedure:

1. Obtain an 8-hour overnight urine specimen.
2. Place the *entire* specimen in an Erlenmeyer flask of convenient size.
3. Add 50 ml. of toluene (reagent quality), 10 volumes per cent of concentrated hydrochloric acid, and a few glass beads to the flask.
4. Connect the flask to a vertical Liebig condenser and reflux vigorously on the hot plate for 15 minutes.
5. Remove the flask and cool rapidly to room temperature under the water tap. Avoid agitation of the sample while cooling.
6. Transfer the sample to a separatory funnel and draw off and discard the lower layer (urine).
7. If only a small amount of emulsion is present at this point it can be disregarded. If a large amount is present, transfer the urine-toluene emulsion to a 100 ml. tube and centrifuge for 5 minutes at 1500 revolutions per minute.
8. Add 15 ml. of 0.1 N sodium hydroxide to the separatory funnel and wash by swirling the sample gently.
9. The sodium hydroxide solution will settle to the bottom and is discarded.
10. Repeat step #8 with an additional 15 ml. of sodium hydroxide and follow the same procedure with two 15 ml. portions of water.
11. After the last washing with water make a very careful separation so that only the toluene layer is transferred to a *dry* 125 ml. Erlenmeyer flask.
12. Allow the sample to stand undisturbed for at least 5 minutes and then pour it into a second dry 125 ml. Erlenmeyer flask making sure that any droplets of water are left behind.
13. Add 2 glass beads and boil on the hot plate.
14. When the vapors reach the mouth of the flask, add slowly 10 ml. of 2% sodium hydroxide in absolute methanol while the flask remains on the hot plate.
15. Boil until the toluene is reduced to about one-half of the original volume.
16. Filter while hot through a fritted glass filter (Pyrex, medium porosity) using suction.
17. Place the filtrate in a dry 125 ml. Erlenmeyer flask and take to dryness on the hot plate. To avoid overheating of the residue use a gentle air stream to remove the last traces of toluene.
18. Add 5 ml. of acetone to the residue.
19. Place the flask on the hot plate and add 25 ml. of boiling 0.1 N sodium hydroxide, slowly, 3 to 4 ml. at a time. After the addition of the first 3 to 4 ml. of the sodium hydroxide solution to the 5 ml. of acetone, reduce the volume to approximately 3 ml. before addition of the next portion. The remainder of the sodium hydroxide may be added more rapidly—shaking the flask and bringing to a boil between the addition of each portion.
20. Remove the sample from the hot plate and observe in a good light. In strongly positive samples a macroscopic, white, flaky precipitate will be seen in the solution and particularly on the side of the flask above the level of the liquid. If a precipitate is present and plainly seen at this stage, record as a four-plus reaction and carry the procedure no further. In case of no precipitate or a questionable result continue with step #21.
21. Place the flask in the refrigerator or an ice bath until cold.

April and May the cycles were 37 and 41 days respectively with normal pregnandiol excretion.

Our studies of pregnandiol excretion in instances of sterility have shown numerous cycles in which absence of the typical biphasic temperature shift

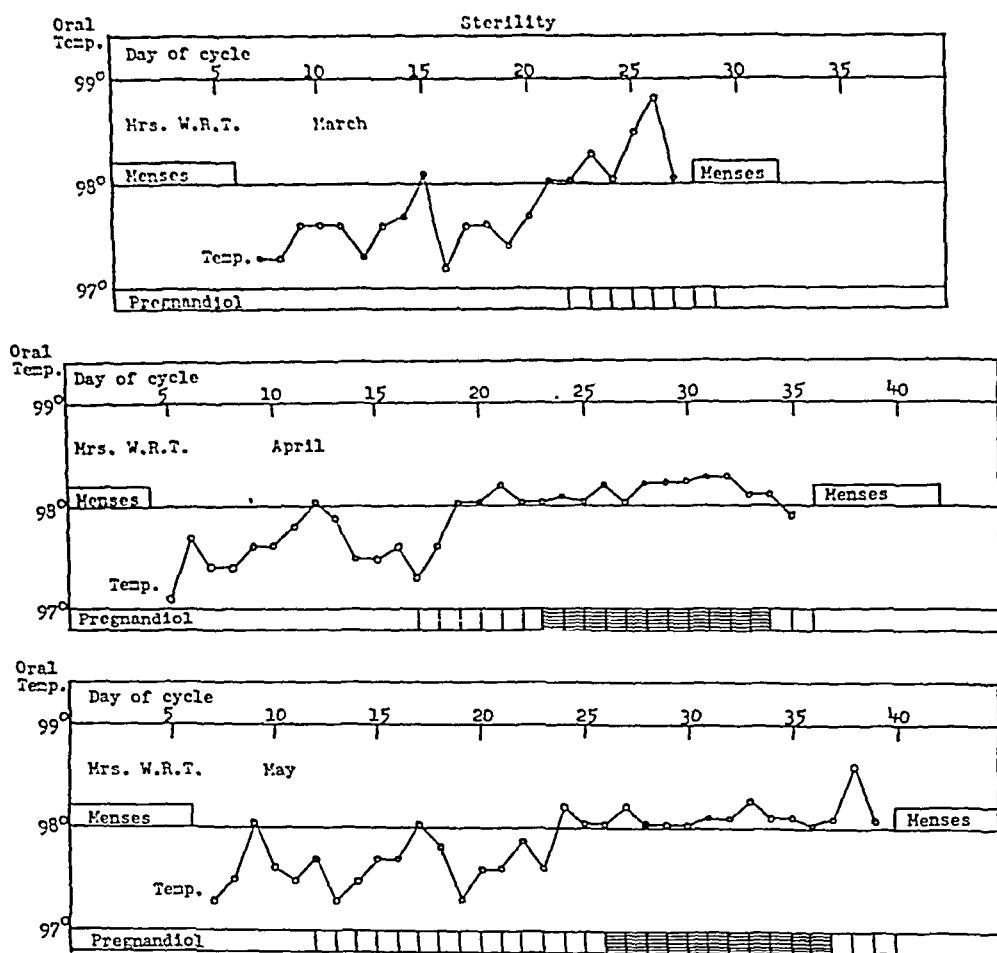


FIG. 4. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

and lack of pregnandiol excretion appear to offer some explanation for infertility on the basis of failure of ovulation. The estimation of pregnandiol in the study of patients with disturbances of menstruation or infertility may thus offer information of considerable diagnostic importance. Detailed analysis of these studies will be presented in a subsequent report.

Care of fritted glass filter (step #16).

The same fritted glass filter has been used continuously for approximately 6000 determinations of pregnandiol performed in this laboratory in the past two years. After each filtration the surface precipitate is washed off under the hot water tap and about 30 ml. of hot water is drawn through the filter by suction. This is followed by a few ml. of 95 per cent ethyl alcohol and lastly 5 ml. of acetone. By following this procedure the filter is left clean and dry for the next filtration. Once a week concentrated sulfuric acid is used to remove any accumulated organic material.

Addition of sodium hydroxide (step #19).

We wish to re-emphasize the importance of slow initial addition of the sodium hydroxide solution in step #19. After addition of the first 3 to 4 ml. of sodium hydroxide the volume is reduced by boiling to a total of approximately 3 ml. before addition of the next portion. The remainder of the solution may then be added more rapidly.

Delayed precipitation.

We have consistently given the impression that the pregnandiol precipitation test can be completed in 1 hour or less. However, this is only true of those samples giving an immediate positive result in step #20 or #24. It has been observed that occasionally a reaction originally interpreted as negative will have become positive upon re-inspection the following morning. Inasmuch as routine samples need not be rushed to completion we place the flasks from step #20 in the refrigerator as they are completed. Before leaving the laboratory at night one ml. of each sample is transferred to a Kahn tube and allowed to remain at room temperature until morning. The results are then read in the Kahn viewing device and recorded as negative, questionable, two-plus, three-plus, or four-plus.

Use of hydrochloric acid in precipitation (step #24).

One drop of concentrated hydrochloric acid is added to tubes showing negative, questionable, or weak positive reactions and after agitating, the tubes are again scrutinized. Some reactions which were read as negative or questionable will now show a precipitate floating on the top of the liquid. These are recorded as one-plus. By the use of acid it has been possible to make the interpretation of the reaction more objective and to reduce the number of false negative results without increasing the number of false positives.

RESULTS

The pregnandiol precipitation test has been employed in our laboratory since May, 1946, in conjunction with the Friedman test on all urine samples submitted for the diagnosis of pregnancy. From May, 1946 to April, 1948, 737 tests have been performed. Following the reports of Guterman (5, 9) concerning the prognostic significance of pregnandiol excretion levels in cases of threatened abortion, we have applied our method to a similar study of 149 patients.

Normal Pregnancy (Table 1). Urine samples from 381 women subsequently found to have been pregnant were submitted for diagnosis. Of these, 361 gave correct positive pregnandiol readings (94.8%); 20 gave incorrect negative readings (5.2%). The Friedman test performed simultane-

22. Transfer 1 ml. of the sample to a Kahn tube and allow it to come to room temperature.
23. Read in the following manner using a Kahn viewing device:¹
 - (a) A "two-plus" reaction shows a very fine precipitate which requires careful scrutiny of the tube to be visible.
 - (b) A "three-plus" reaction is still a fairly fine precipitate but is readily evident upon looking at the tube in the viewing box.
 - (c) A "four-plus" reaction is reserved for those reactions which show an agglutination of the precipitate into large particles, or for those which were positive in step #20.
24. Add one drop of concentrated hydrochloric acid to tubes which still show a negative or questionably positive reaction. Some of these now show a precipitate which floats on the surface of the liquid. Record this as a "one-plus" reaction.

Reagents

1. 0.1 *N* sodium hydroxide (approximately):—Prepare a stock solution of 2 *N* sodium hydroxide by making 160 grams of sodium hydroxide pellets C.P. up to 2 liters with distilled water. For approximately 0.1 *N* sodium hydroxide, make 100 ml. of the stock solution up to 2 liters with distilled water. These solutions are not critical. No further standardization is necessary.
2. 2% sodium hydroxide in absolute methanol:—Dissolve approximately 2 grams of sodium hydroxide pellets C.P. in 100 ml. of absolute methanol (synthetic or C.P.). Prepare fresh daily and filter before use.
3. Hydrochloric acid C.P.
4. Toluene (purified or reagent quality).
5. Acetone C.P.
6. Methyl alcohol, absolute C.P.

MODIFICATIONS

Eight-hour specimen (step #1).

We originally followed Guterman's example (5, 6) in using 100 ml. of a first morning specimen of urine for assay. It was soon evident, however, that a more constant aliquot of the 24-hour urine excretion was necessary, since overnight samples varied from 50 to 1500 ml. Since, also, the daily pregnandiol output during the luteal phase of the cycle and early pregnancy has been shown to vary from 1.5 to 10 mg. (7), and since our technic accurately detects 0.5 mg. of pregnandiol (1), consistently positive results in women excreting a minimum of 1.5 mg. per day could not be expected unless one-third of the daily output was analyzed. Accordingly, we now insist upon 8-hour samples. The consistent use of 8-hour specimens makes it possible to compare the amounts excreted by different women or by the same woman from day to day. Somerville, Gough, and Marrian (8) also found 100 ml. samples of urine inadequate for pregnandiol assay and suggested using one-fifth of a 24-hour specimen.

¹ Sell, Floyd: "Viewing Device for the Final Stage of the Kahn Test." Presented before the Illuminating Engineering Society, Great Lakes Regional Conference, June 5, 1943.

subsequent reports from physicians who submitted the samples for diagnosis.

Nonpregnant states (Table 2). Urine samples from 356 patients subsequently determined not to have been pregnant at the time of testing gave correct negative pregnandiol reactions in 316 (88.8%); false positive tests were reported in 40 (11.2%). The Friedman test gave correct negative reactions in 277 of 282 tests (98.2%). There were 5 false positive Friedman reactions (1.8%).

False positive pregnandiol tests (Table 3). The case histories of patients with false positive pregnandiol reactions were subjected to further scrutiny after the clinical results became known. The 18 patients classified as "delayed menses" had gone more than seven days beyond the date of the expected period when the tests were made; 13 of these patients always menstruated at irregular intervals. The possibility of late ovulation and consequently delayed menstruation in such instances has been illustrated

TABLE 3

FALSE POSITIVE PREGNANDIOL TESTS

No amenorrhea	18
Delayed menses	18
Corpus luteum cyst	2
Lactating	1
Secondary amenorrhea	1
<hr/>	
Total	40

in Figures 3 and 4. In 18 of the 40 cases, specimens were submitted during the luteal phases of normal cycles because of pelvic findings (tumor, adnexal enlargement) suggesting to the physician the possibility of pregnancy, or because of abnormal flow during the preceding cycle. In two instances positive tests were obtained in patients operated upon for corpus luteum cysts.

It is evident from the foregoing and from other reports in the literature (6, 10, 11) that the positive pregnandiol test should be interpreted with caution since it gives presumptive evidence of pregnancy *only when the urine is obtained during periods of amenorrhea in women with otherwise normal menstrual cycles*. When these criteria are followed, the accuracy of the pregnandiol test in nonpregnant states compares favorably to that in normal pregnancy. In cycles regularly surpassing the usual 28-day length (Figs. 3 and 4), late pregnandiol excretion as a consequence of late ovulation may be expected. These fundamental facts cannot be over-emphasized since lack of their understanding has led to false interpretations of the test. When cycles are consistently irregular, the Friedman test should be performed simultaneously.

TABLE 1

Duration of amenorrhea (from expected menstrual period to time of test)	<i>Normal pregnancy</i>			
	Pregnandiol precipitation test		Friedman test	
	False negative	Correct positive	False negative	Correct positive
Less than 1 week	1	18	3	16
1 to 2 weeks	4	60	1	57
2 to 3 weeks	3	46	1	44
3 to 4 weeks	3	46	3	41
Over 4 weeks	6	132	3	117
Unknown	3	59	2	58
	—	—	—	—
	20	361	13	333
	(5.2%)	(94.8%)	(3.8%)	(96.2%)
	Total 381		Total 346	

TABLE 2

Condition	<i>Nonpregnant states</i>			
	Pregnandiol precipitation test		Friedman test	
	False positive	Correct negative	False positive	Correct negative
Delayed menses	18	59	1	70
Secondary amenorrhea	1	11	0	12
Functional bleeding	0	14	1	10
Menopause	2	13	0	13
Postabortion	0	18	0	8
Lactation	1	5	0	6
Pelvic inflammatory disease	1	25	0	3
Fibroids	3	20	0	12
Ovarian cysts and tumors	4	8	0	7
Pseudocyesis	0	3	0	0
Insufficient data	10	140	3	136
	—	—	—	—
	40	316	5	277
	(11.2%)	(88.8%)	(1.8%)	(98.2%)
	Total 356		Total 282	

ously (in instances where sufficient urine was available) gave correct positive reactions in 333 of 346 specimens (96.2%); false negative tests were obtained in 13 (3.8%). The accuracy of these tests was determined by

pregnant could be explained in part by delayed pregnandiol excretion consequent to late ovulation in irregular cycles; or by the urine specimen having been procured during the luteal phases of normal cycles. When these cases are eliminated and the test is restricted to instances of genuine amenorrhea, the accuracy in nonpregnant states is 97.2 per cent and it thus compares closely to that obtained during normal pregnancy. *Pregnandiol excretion offers presumptive evidence of pregnancy only during amenorrhea of previously normally menstruating women.* When cycles are consistently prolonged or irregular, the Friedman test should be performed in conjunction. The Friedman test showed an accuracy of 96.2 per cent in normal pregnancy and 98.2 per cent in nonpregnant states.

The prognostic value of the pregnandiol precipitation test in threatened abortion has been investigated in 149 cases. Of 53 patients giving negative test results, 51 (96.2 per cent) aborted. From this it seems evident that negative pregnandiol tests during pregnancy are practically pathognomonic of subsequent abortion.

Preliminary investigations of pregnandiol excretion during the postovulatory phases of menstrual cycles, in conjunction with basal temperature determinations, indicate further usefulness of the test in the study of problems of infertility.

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TABLE 4

THREATENED ABORTION						
Retention of fetus—68 patients						
No. of weeks gestation	5-8	9-12	13-16	17-20	Unknown	Total
Pregnandiol test positive	23	28	8	7	0	66
Pregnandiol test negative	2	0	0	0	0	2
Abortion—81 patients						
No. of weeks gestation	5-8	9-12	13-16	17-20	Unknown	Total
Pregnandiol test positive	8	11	7	3	1	30
Pregnandiol test negative	14	30	2	4	1	51

Threatened abortion. Table 4 shows the results of a study in 149 patients with symptoms of threatened abortion (cramps, bleeding) during early pregnancy. In 51 cases, negative pregnandiol tests preceded the occurrence of spontaneous abortion. In 10 instances, positive pregnandiol tests became negative during the period of observation in cases which similarly terminated in fetal loss. In 66 cases, pregnandiol tests remained positive during the period of symptoms in cases in which the fetus was retained. Incorrect prediction was made in 30 cases in which the tests remained positive despite eventual abortion. Nineteen of the 30 cases were in the first trimester of gestation when abortion took place. The possibility that positive tests in some instances were associated with nonendocrine causes of abortion must also be considered. However, while the pregnandiol precipitation test evidently provides rapid screening, it may be necessary to resort to quantitative methods for the detection of decreasing pregnandiol levels in instances where persistent positive reactions are obtained despite continuation of symptoms.

The qualitative pregnandiol precipitation test has its greatest value during threatened abortion prior to the fifth month of gestation. Since increasing placental function adds greatly to the output of pregnandiol after the fourth month it may also be necessary to rely upon quantitative methods to determine diminished progesterone metabolism in later stages. Of the 53 patients giving negative results with this test, 51 or 96.2 per cent aborted. From these data it seems evident that in pregnancy negative pregnandiol tests are almost pathognomonic of subsequent abortion.

SUMMARY AND CONCLUSIONS

The pregnandiol precipitation test, performed in 737 urine specimens submitted for the rapid diagnosis of pregnancy showed an over-all efficiency of 91.9 per cent. In cases of normal pregnancy the accuracy was 94.8 per cent, whereas in nonpregnant states it was 88.8 per cent. False positive tests reported in women subsequently found not to have been

TABLE 2. PATIENTS WITH DIABETES MELLITUS WHO SUBSEQUENTLY DEVELOPED ADDISON'S DISEASE

Author*	Patient's		Duration of diabetes at time of onset of Addison's disease yrs.	Apparent etiology of Addison's disease	Duration of Addison's disease
	Age yrs.	Sex			
Unverricht (7) 1926	32	M	6	Tuberculosis	2-4 months
Rowntree & Snell (8) 1931	25	M	1	Atrophy	15 months
Rowntree & Snell (8) 1931	—	M	6	Atrophy	18 months
Rogoff (9) 1936	25	M	4	Denervation of adrenals	12 months
Bloomfield (10) 1939	30	M	1	? Atrophy	Patient alive
Bowen, <i>et al.</i> (11) 1942	76	F	10	Tuberculosis	12 months
McCullagh (12) 1942	—	M	7	? Atrophy	Patient alive
Bickel (13) 1945	29	M	6	?	Patient alive
Devitt & Murphy (14) 1947	28	F	3½	Atrophy	3 months
Adler (15) 1947	20	F	2	? Atrophy	Still living

* Since this article was written, we have the following references to add to this category:

Allan (16) 1930—a review only.

Brookfield and Corbett (17) 1934—diagnosis of Addison's disease questionable.

Heim (18) 1940.

Sprague *et al.* (19) 1947—three cases, reported in a brief abstract.

TABLE 3. THOSE PATIENTS IN WHOM ADDISON'S DISEASE PRECEDED DIABETES MELLITUS

Author*	Patient's		Duration of Addison's disease prior to diabetes mellitus years	Etiology of Addison's disease	Duration of combined diseases
	Age yrs.	Sex			
Rhind & Wilson (20) 1941	32	F	2	Atrophy	2 mo.—died
Thorn & Clinton (2) 1943	23	M	4½	Unknown	Alive
Soffer & Sorkin (21) 1945	42	M	2	Unknown	Alive

* Since this article was written, we have the following references to add to this category:

Lowrie *et al.* (22) 1948.

Knowlton and Kritzler (23) 1949.

CARBOHYDRATE METABOLISM IN THE COMBINATION OF DIABETES MELLITUS AND ADDISON'S DISEASE, AS ILLUSTRATED BY A CASE

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SIMULTANEOUS occurrence of Addison's disease and diabetes mellitus is rarely observed. It is surprising that this is only the nineteenth report of a proven occurrence of these two diseases in the same patient, when 28.4 per cent of patients with diabetes mellitus also have active tuberculosis (1). We have combined those cases summarized by Thorn and Clinton in 1943 (2) with the subsequent case reports in Tables 1, 2, and 3.

In both Addison's disease and diabetes mellitus there are marked aberrations in carbohydrate metabolism. A better understanding of these diversions in the individual disease and realization of the characteristic pattern of their summation should lead to premortem diagnosis of adrenocortical insufficiency in a greater number of diabetic patients. To clarify this pattern, a synthesis is presented to illustrate the various abnormalities in carbohydrate and related metabolism. Special emphasis is placed on the biochemical and clinical modifications appearing in each disease.

TABLE 1. PATIENTS WITH SIMULTANEOUS ONSET OF DIABETES MELLITUS AND ADDISON'S DISEASE

Author		Patient's		Duration of disease yrs.	Pathologic findings in adrenal gland	Insulin requirements
		Age yrs.	Sex			
Arnett	(3) 1927	39	F	3	Atrophy	20-50 units daily
Levy-Simpson	(4) 1932	16	M	1	Atrophy	10 units resulted in hypoglycemia
Gowen	(5) 1932	54	F	1-5	Atrophy	5 units resulted in hypoglycemia
Koepf & Bowen (unpublished)	(2)	20	M	2	Atrophy	diabetes controlled by diet
Nix	(6) 1943	39	M	1½	Atrophy	not controlled

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materials from the gut, secondary to the lack of salt-active hormones of the adrenal cortex.

Clinically, these alterations are characterized by: a) extreme insulin sensitivity, b) fasting hypoglycemia, c) flat oral glucose tolerance curve, d) an intravenous glucose tolerance curve which shows a normal initial rise and then a fall to moderate or severe hypoglycemic levels, e) hypoglycemic reactions which tend to be more severe at higher levels of the blood sugar than in the normal individual (37), and f) a failure of epinephrine to produce a normal rise in the blood sugar (37).

Protein metabolism is significantly changed in Addison's disease. In untreated chronic adrenocortical insufficiency, conversion of protein into glucose diminishes (35). Conversely, it is noted that after administration of certain fractions (Kendall compounds A, B, and E) of adrenocortical extract, excretion of nitrogen in the urine increases, denoting accelerated protein catabolism (38).

In Addison's disease impairment of fat metabolism is shown by: a) diminished ability to utilize the intermediate products of fatty acid metabolism (36); b) lessened formation of ketone bodies after injection of phlorrhizin (39); and c) inability to deposit fat in the liver (40). It should be noted that the tendency toward acidosis often found in Addison's disease may increase when diabetes mellitus supervenes. This is probably the result of two factors: diminished ability to utilize the intermediate products of fatty acid metabolism, and excessive excretion of sodium by the kidneys due to lack of salt-active adrenal hormones.

The case to be presented illustrates the interaction of the many and diverse physiochemical processes described above.

CASE HISTORY

First Admission: On July 8, 1942, L. R., a 56-year-old woman, was referred to the Mason Clinic for control of diabetes mellitus of sixteen years' duration, for which she had been taking crystalline insulin (C), 20 units, before each meal. The past history disclosed that in 1920 a goiter was removed because of symptoms of toxicity. Her general state of health had remained good.

Physical examination revealed a blood pressure of 108 systolic and 68 diastolic, a dry and sallow skin with a vague bluish tint; there were no other significant physical findings. The urine showed a 2-plus sugar and 1-plus albumin. The blood hemoglobin was 14 Gm.; erythrocyte count, 5,000,000; and leukocyte count, 7,300, with a normal differential count. The blood Kahn and Kolmer tests were negative. Blood sugar levels ranged from 108 to 385 mg. per cent. A roentgenogram of the chest demonstrated calcified mediastinal lymph nodes.

The patient was discharged on a diet of 175 Gm. of carbohydrate, 80 Gm. of protein and 100 Gm. of fat; and was to take 20 units of protamine zinc insulin (PZI) and 10 units of crystalline insulin (C-10) before breakfast.

Interval: Ten months later she returned complaining of mild nausea. This continued

Carbohydrate metabolism in diabetes mellitus. Diabetes mellitus, fundamentally, results from a deficiency of the hormone insulin. Cori and Cori (24) have recently centered attention upon the enzyme hexokinase and its probable role in facilitating the reaction: glucose plus adenosine triphosphate yields glucose-6-phosphate plus adenosine diphosphate. This reaction represents the first step in the body's utilization of glucose. It is inhibited by the diabetogenic hormone of the anterior pituitary. Insulin acts as an antagonist to the diabetogenic hormone, and has no effect in its absence (25). Therefore, the apparent and perhaps sole effect of insulin is to augment this reaction.

A deficiency of insulin leads to the following secondary biochemical alterations in carbohydrate metabolism: a) increased glycogenolysis, b) decreased rate of utilization of glucose in muscle (26) (the rate approaches normal only at high concentrations of glucose (27)), and c) increased gluconeogenesis (28). Clinically, these are manifested by: a) hyperglycemia, b) glycosuria, and c) glucose tolerance curves with a rapid rise and prolonged elevation in the blood sugar level.

Controlled diabetes mellitus has no specific effect upon protein metabolism, but protein catabolism is increased during uncontrolled periods by the impaired capacity of the organism to utilize glucose and by the resulting hyperglycemia and glycosuria. This chain of events leads to accelerated gluconeogenesis from protein, demonstrated by a negative nitrogen balance and increased urinary nitrogen excretion (29).

The frequency of lipemia in diabetes mellitus indicates alterations in fat metabolism. The exact mechanism involved is unknown, but it probably results from increased mobilization of body lipids secondary to conversion of lipids to glucose. Also, diminished lipogenesis from carbohydrate has been demonstrated (30). Clinically and pathologically these changes are manifested by: a) ketonemia, b) ketonuria, c) excessive deposition of fat in the liver, and d) lipemia.

Carbohydrate metabolism in Addison's disease. In Addison's disease the principal defect in carbohydrate metabolism is the inability to derive glucose from endogenous and exogenous sources. Adrenal cortical hormones have been shown to have an inhibitory effect upon the hexokinase reaction similar to that of the diabetogenic hormone of the anterior pituitary (24). This defect is represented by: a) diminished conversion of glucose to glycogen in both liver and muscle (31, 32); b) no decrease in the utilization of glucose in muscle (33, 34); c) decreased gluconeogenesis from protein (35) and fat (36); d) decreased rate of conversion of glycogen to glucose; and e) reduced absorption of glucose from the gastro-intestinal tract (37). The last phenomenon is probably the result of changes in physical absorption of

of adrenal hormone therapy, there was progressive deterioration to her former state. Again this was relieved almost immediately by intensive treatment with intravenous saline, DCA, and adrenocortical extract. She was well enough to be discharged seventy-two hours after the reinstitution of therapy. Her discharge diet was carbohydrate 165 Gm., protein 80 Gm., and fat 90 Gm. She was instructed to take daily, 7 mg. of DCA normal dietary salt, 3 Gm. of supplementary salt, and PZI, 8 units.

Interval: One month later, because of progressive weight increase and edema of the ankles, the DCA was reduced to 3 mg. daily and the salt was discontinued. Apathy, nausea, vomiting, malaise and weakness soon reappeared and progressed, even though the dosage of DCA was increased to 6 mg. daily.

Third Admission: November 26, 1946 (four months after second admission). She was unable to give a coherent story. There was an increase in skin pigmentation and, for the first time, a dusky hue of the oral mucous membrane was observed. Her blood pressure was 155 systolic and 100 diastolic. There was no evidence of heart failure or edema.

The blood hemoglobin was 12 Gm.; erythrocyte count, 3,600,000; and the leukocyte count, 5,750. Urinalysis showed a few leukocytes and a slight amount of albumin. The blood chloride was 429 mg. per cent; nonprotein nitrogen, 25 mg. per cent; serum sodium 315 mg. per cent; and serum protein, 5.01 Gm. per cent, with an albumin-globulin ratio of 2.01. The DCA was increased from 6 to 12 mg. In addition, 20 to 30 cc. of aqueous adrenocortical extract and 18 Gm. of sodium chloride were given intravenously daily. During the last four days of life this was supplemented each day with 4 to 6 cc. of lipo-adrenal extract, given intramuscularly. Throughout she received PZI, 8 units each morning before breakfast.

The blood pressure remained between 160 systolic and 80 diastolic, and 170 systolic and 100 diastolic. Her urinary output was normal, and there was no evidence of fluid retention. Despite seemingly adequate therapy, the patient did not become mentally alert nor did she take food. She went progressively downhill, and died without terminal incident on December 7, 1946, twenty years after the onset of diabetes, and three years and seven months after the onset of Addison's disease.

Necropsy Findings: (Only pertinent findings will be presented). The heart weighed 335 Gm. The myocardium was of normal thickness and no areas of fibrosis were noted.

The kidneys weighed 310 Gm. The surfaces were finely granular. On microscopic section, there was a marked increase in the interstitial connective tissue and a rather extensive atrophy of the tubules. The latter contained many hyaline casts. The glomeruli showed extensive hyalinization and occasional epithelial crescents.

The liver was normal in gross appearance, on cut section, and on microscopic study. Stains for hemosiderin gave negative results.

In the pancreas only increased fibrosis was found.

Each adrenal gland weighed less than 5 Gm. On cut section, the normal architecture was completely lost. Microscopically, the adrenal was almost entirely replaced by an acute tuberculous process, represented by scattered Langhans' giant cells and diffuse epithelioid cell formation. A few areas of typical cortical cells could be distinguished.

Pathologic diagnoses were: 1) tuberculosis of the adrenal glands, and 2) chronic glomerulonephritis.

DISCUSSION

In 18 of the cases previously reported, diabetes mellitus preceded the onset of Addison's disease in 10; the reverse occurred in only 3 instances and the onset was simultaneous in the remaining 5. However, virtually the

intermittently over a period of seventeen months despite minor adjustments in insulin dosage. Three years and four months after discharge she contracted mumps. She was hospitalized elsewhere and given 20 units of globin insulin daily. Later, afternoon hypoglycemic reactions were noted, even though the patient decreased the globin insulin to 12 units daily. At the same time, she experienced progressive weakness, anorexia, weight loss (8 pounds), increasing malaise, intermittent diarrhea, and deepening facial pigmentation. However, she continued to work until vomiting started two days before readmission.

Second Admission: July 28, 1946 (four years after first admission). The patient appeared chronically ill and weak. A dirty brownish pigmentation of the face and shoulders without discoloration of the mucous membrane of the mouth or the breast areolae was noted. The blood pressure was 110 systolic and 70 diastolic, and the pulse rate was 80.

Urinalysis showed a moderate number of leukocytes. The complete blood count was normal. The blood chloride (expressed as sodium chloride) was 263 mg. per cent; the sugar, 504 mg. per cent; the cholesterol, 400 mg. per cent; and the carbon dioxide combining power, 60 volumes per cent. The bromsulphalein liver function test (5 mg. per Kg.) showed less than 5 per cent retention in forty-five minutes. A roentgenogram of the abdomen revealed a small calcification just to the left of the transverse process of the second lumbar vertebra. X-ray studies of the stomach, duodenum, and gall bladder showed these organs to be normal. Basal metabolic rates were minus 21 and minus 30 per cent. An electrocardiogram demonstrated a rate of 86, a PR interval of 0.19, a low R-1, inverted T-2 and T-3, isoelectric T-4 and a flat T-1. The voltage was at the lower limits of normal.

Extreme weakness, malaise, and intolerance to food with nausea and vomiting continued. She was too weak to rise from the supine position. Many mornings she was found in a comatose state from which she responded only after the administration of glucose in physiologic saline. The systolic blood pressure ranged from 88 to 54. The blood sugar during one such episode was 91 mg. per cent.

At first the patient was maintained on intravenous fluids. Then, after a short interval of aqueous adrenocortical extract administration, she was given 4 mg. daily of desoxycorticosterone acetate (DCA) in oil, intramuscularly, supplemented by 3 Gm. of sodium chloride orally. She remained somewhat nauseated; therefore the DCA was increased to 7 mg. On this regimen she had no nausea or vomiting, and her appetite was excellent. She became ambulatory, and was bright and alert, in marked contrast to her previous lethargy.

As soon as DCA therapy was begun, she started to have severe hypoglycemic reactions before and after breakfast. Sugar determinations on blood obtained before breakfast were near 50 mg. per cent. Her diabetes mellitus had been controlled on PZI, 12 to 16 units daily, but hypoglycemic reactions which followed treatment with DCA forced a reduction to a maintenance dose of PZI, 8 units daily. After one week on this regimen the blood pressure rose to 135 systolic and 80 diastolic, and the blood chlorides to 462 mg. per cent. Reversion toward normal was noted in the electrocardiogram.

In an effort to establish the diagnosis more conclusively, DCA was withdrawn and placebo hypodermic injections were substituted. Salt was withdrawn from the diet and none given additionally. At the end of sixty hours the Robinson-Power-Kepler water diuresis test was performed. The day excretion of urine equalled the night excretion, and the final equated figure was 23. A sodium determination on serum taken during this interval was 310 mg. per cent (the normal blood sodium in our laboratory is 330-340 mg. per cent), and the blood chlorides were 478 mg. per cent. During the withdrawal

no insulin was given. In a similar manner, our case illustrates a rather striking fall in blood sugar and insulin requirement with the institution of desoxycorticosterone acetate therapy. There was a marked tendency toward hypoglycemic reactions previously not present when this drug was being administered. It should be emphasized that increased insulin sensitivity is to be expected in the treatment of the two diseases when desoxycorticosterone acetate is employed.

Diminution of any magnitude in insulin requirement in diabetes mellitus is unusual, once that requirement has been established. Such a diminution should suggest possible intercurrent of Addison's disease, although similar changes may be noted with the onset of liver disease (43), hypothyroidism (44), hypopituitarism (45), and congestive heart failure in the diabetic (46).

SUMMARY

1. A case of diabetes mellitus with superimposed Addison's disease is presented. This is the nineteenth such patient reported in the literature.

2. Carbohydrate and related metabolic alterations have been discussed in reference to diabetes mellitus, chronic adrenocortical insufficiency, and the combination of these two diseases.

3. In contrast to the opinion of many authors, the administration of desoxycorticosterone acetate appears to cause definite changes in the carbohydrate metabolism.

4. In the presence of a progressively diminishing insulin requirement in a diabetic, the diagnosis of Addison's disease should be considered.

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same clinical and laboratory picture eventually prevailed in all. Progress of the patient described here illustrates the typical modifications in the carbohydrate metabolism of the diabetic, brought about by Addison's disease. In short, diabetes mellitus is attenuated by the onset of Addison's disease, as Long and Lukens demonstrated experimentally (41).

The following changes, briefly summarized, noted in our patient and in those previously described, are: a) Because glycogeneolysis increases in diabetes mellitus and glycogen formation decreases in Addison's disease, both diseases, either separately or together, lower liver and muscle glycogen. b) Decreased utilization of glucose in the tissues is noted in diabetes mellitus. This is not true in Addison's disease and, in fact, such utilization may increase. In combination, these two effects result in a decreased utilization of glucose which is probably not as great as that found in diabetes mellitus alone. c) Although gluconeogenesis increases when the two diseases occur together, it is not enhanced to the extent that it is in uncomplicated diabetes mellitus (37).

Clinically, these basic processes are manifested by: a) decrease in blood sugar level and decrease in glycosuria with the onset of Addison's disease; b) progressive decrease in the amount of insulin necessary for adequate control of the diabetes mellitus, and increased insulin sensitivity; and c) hypoglycemic reactions at higher levels of the blood sugar.

Kendall (38) has established the relative values of the chemical fractions of the adrenal cortex related to carbohydrate metabolism. The most important of these are: a) 11-dehydro-17-hydroxycorticosterone (Compound E); b) 11-dehydrocorticosterone (Compound A); and c) corticosterone (Compound B). At present, desoxycorticosterone acetate is the only adrenal steroid commercially available. In most cases, it gives adequate control when there is a high carbohydrate intake. It has been generally assumed that desoxycorticosterone acetate has no effect upon carbohydrate metabolism in chronic adrenocortical insufficiency. However, Loeb (42) has noted that hypoglycemia appears to occur more frequently in patients treated with salt and desoxycorticosterone acetate than in untreated patients. Bloomfield (10) has conclusively demonstrated that the blood sugar (fasting) decreases in the combination of diabetes mellitus and Addison's disease from a range of 188-76 mg. per cent to a range of 74-50 mg. per cent, when the patient is receiving 5 to 25 mg. of desoxycorticosterone acetate daily. In his patient this process could be repeated at will by withdrawal and reinstitution of the synthetic hormone. The insulin requirements of Bickel's case (13) dropped from PZI 50 units to PZI 10 units at the onset of Addison's disease and, with administration of desoxycorticosterone acetate, the patient showed complete freedom from glycosuria when

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not be palpated. Genital development and secondary sexual characteristics, such as hair distribution, were normal.

Several hypoglycemic crises with the blood sugar between 30 and 47 mg. per cent were observed. With carbohydrate and protein-rich meals given at short intervals (not longer than three hours) these crises were prevented. Sugar tolerance tests (Exton-Rose and five-hour blood sugar curve) gave results similar to those found in hyperinsulinism (Figs. 1 and 2).

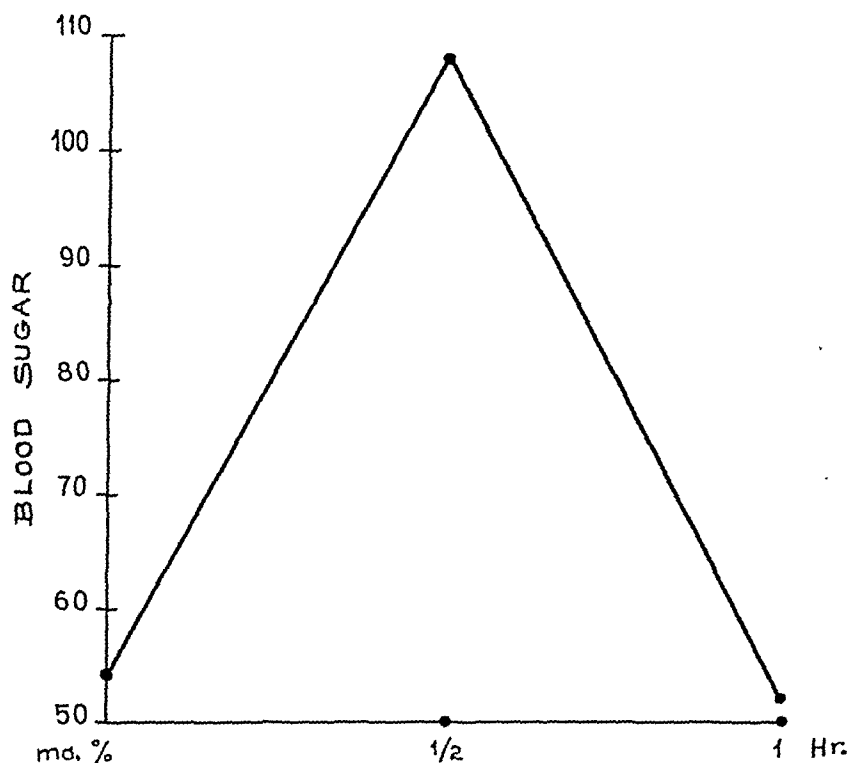


FIG. 1. Exton-Rose sugar tolerance test, before operation.

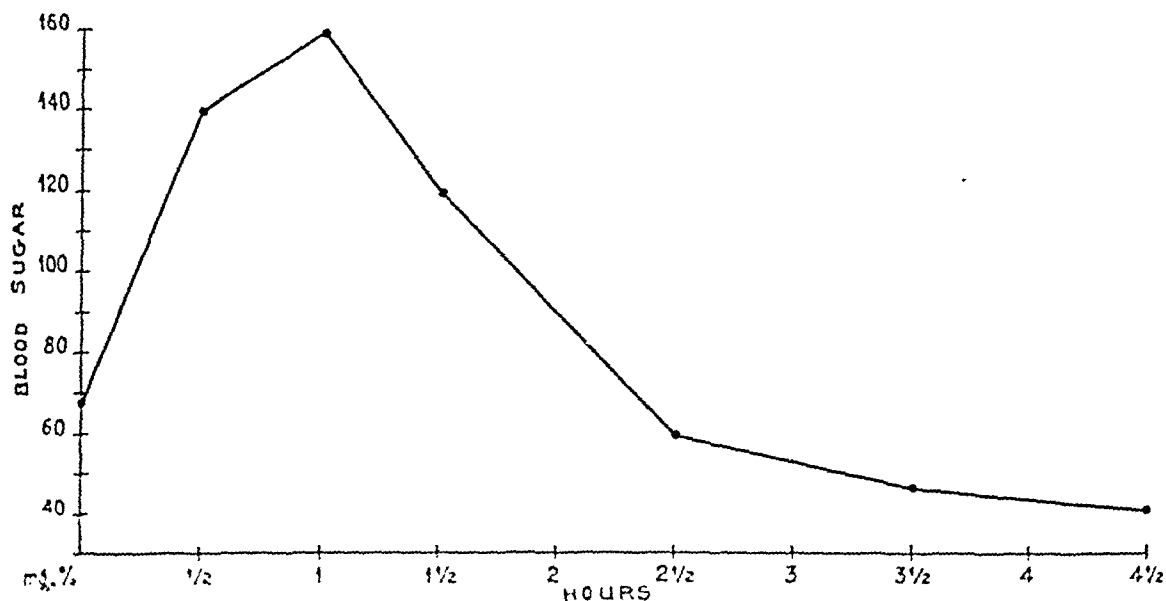


FIG. 2. Prolonged five-hour sugar tolerance test, before operation.

CORTICOADRENAL TUMOR WITH HYPOGLYCEMIC SYNDROME, GOITER, GYNECOMASTIA AND HEPATOSPLENOMEGALY

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THE most frequent disturbance in carbohydrate metabolism observed in cases of corticoadrenal tumor is a diminished tolerance to glucose, with hyperglycemia. It is therefore interesting to record a case of corticoadrenal tumor in which typical hypoglycemic crises occurred repeatedly, without any other sign of Addison's disease. The clinical diagnosis was hyperinsulinism, thought to be caused by a pancreatic new growth, but the large tumor removed at operation was revealed by the pathologic examination to be a corticoadrenal tumor of embryonic type. The thyroid, the liver and the spleen were enlarged, and bilateral gynecomastia was present.

A preliminary report of this case was published in December 1947 (1). Later Broster and Patterson (2) reported a case of corticoadrenal tumor in a young female with intense virilization and hypoglycemic crises; but no mention is made of enlargement of the thyroid, liver and spleen.

CASE REPORT

A. N., was a 25-year-old, single, house painter. Two months before coming to our attention, being in apparently normal health, he could not be awakened one morning. The doctor who was called found him bathed in sweat, more or less awake, and mentally confused. Sweetened orange juice was given and he rapidly recovered. A similar crisis occurred before breakfast four or five times at increasingly shorter intervals. Determinations made during the crises showed blood sugars below 30 mg. per cent, and a rapid return to normal after injecting glucose solution. The first morning in the hospital he was awakened with difficulty and was mentally confused, excited, stammering incoherently and sweating profusely; the blood sugar was 24 mg. per cent. A few minutes after the ingestion of sweetened milk he was again normal.

Physical examination revealed a diffusely enlarged and hard thyroid, without signs of inflammation. The patient had noted this enlargement four months before the date of examination. There was moderate, painful, bilateral gynecomastia. The liver was enlarged, its lower border being palpated 6 cm. below the ribs; its surface was smooth and painless on palpation. The spleen was enlarged on percussion and the lower pole could be felt on palpation. In the right flank a tumor the size of a large grapefruit with a lobulated, irregular surface, was felt. Its upper extremity could not be clearly separated from the liver; its posterior aspect was in contact with the lumbar region and it followed the respiratory movements. It appeared to be an enlarged kidney. The left kidney could

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caused by a tumor situated outside the gastro-intestinal tract, which was otherwise normal.

The patient was operated on under ether anesthesia. The pancreas was carefully explored and found to be macroscopically normal. No aberrant pancreas was found. The liver was enlarged and the kidneys were apparently normal. A large tumor adherent to the right kidney was removed together with the kidney (Fig. 3).

Postoperative course: The patient made a good recovery. Desoxycorticosterone acetate (10 mg.) was injected as a precaution against acute corticoadrenal insufficiency and saline and glucose were injected by the drip method.

From the third day on, the blood pressure remained stable at the normal level. The blood sugar did not fall below 100 mg. during the first forty-eight hours, and remained normal after glucose injection was discontinued on the sixth day. Hypoglycemic crises did not occur after operation.

The thyroid commenced to decrease immediately after the operation, was reduced to

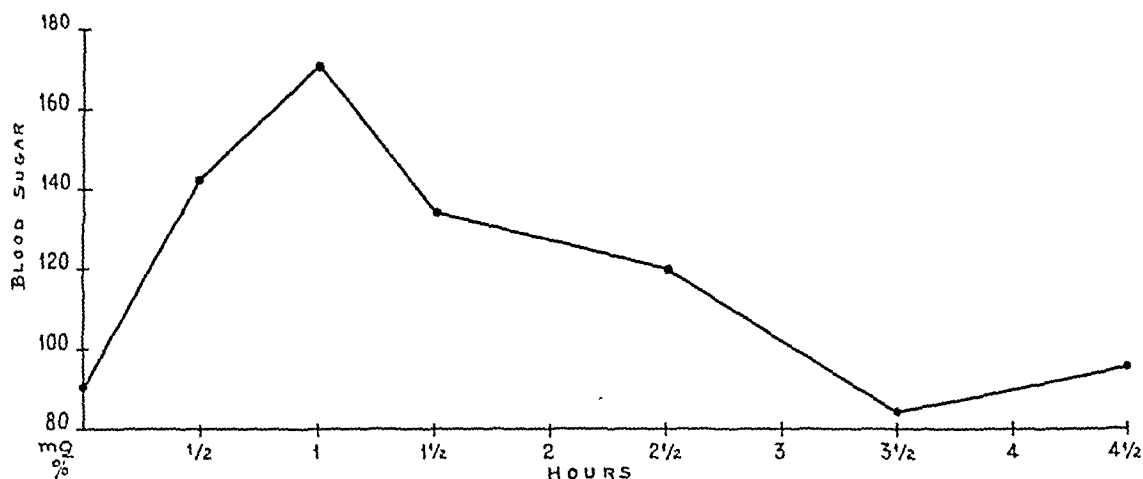


FIG. 4. Prolonged five-hour sugar tolerance test, after operation.

half its size and was much softer by the sixth day. Gynecomastia also retrogressed; by the sixth day it had disappeared completely on the right side and was considerably reduced on the left side. Two weeks later the patient left the hospital in excellent condition.

Deep radiotherapy was given over the area previously occupied by the tumor.

Two months after leaving the hospital the patient came in for a complete examination. The thyroid was considerably reduced in size and hardness. Gynecomastia had disappeared completely. The liver and spleen remained enlarged but at a later examination performed after another interval of three months, these organs had returned to the normal size. The patient was again carefully examined every three months up to two years after the operation, during which time he has remained without relapse and in all respects is apparently normal. The sugar tolerance curve (Fig. 4) confirmed the complete recovery.

Pathologic examination: A rapid microscopic examination of the tumor made during the operation showed it consisted of malignant, atypical tissue, but did not permit the identification of its origin.

The tumor had an ovoid form, $20 \times 9 \times 11$ cm. It formed a single mass, attached to the

The urinary excretion of 17-ketosteroids was 23.24 mg. per day. The hippuric acid test (intravenous) showed an excretion of 1 gram. Blood cholesterol was 190 mg. per cent and the prothrombin test plasma clotting time was 11 seconds. The basal metabolic rate was plus 13 per cent and the electrocardiogram was normal.



FIG. 3. Section of the tumor, showing its relation to the kidney.

A complete x-ray examination was made. The sella turcica was normal. The urinary tract was explored by injection of a contrast substance. The right renal pelvis and ureter were found to be displaced towards the midline. The pelvis on each side was deformed, the calices were lengthened and abnormally separated as is seen in cases of polycystic kidney. The renal shadows were enlarged. The results of intra-ureteral injection confirmed these findings. The stomach and duodenum were found displaced towards the left and the right angle of the colon was at a subnormal level. This displacement was

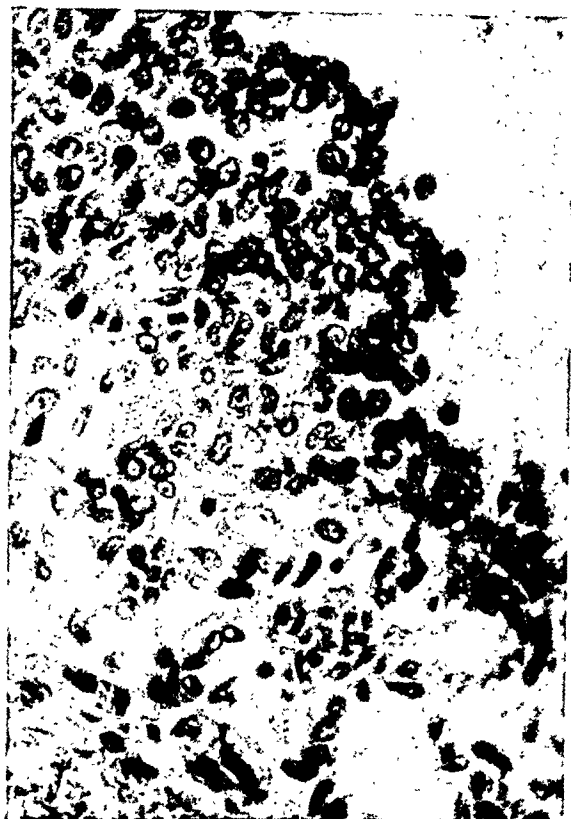


FIG. 6. Cavity formed in a dense zone. Note continuity of the cells in this zone with the lax perivascular tissue. $\times 400$.

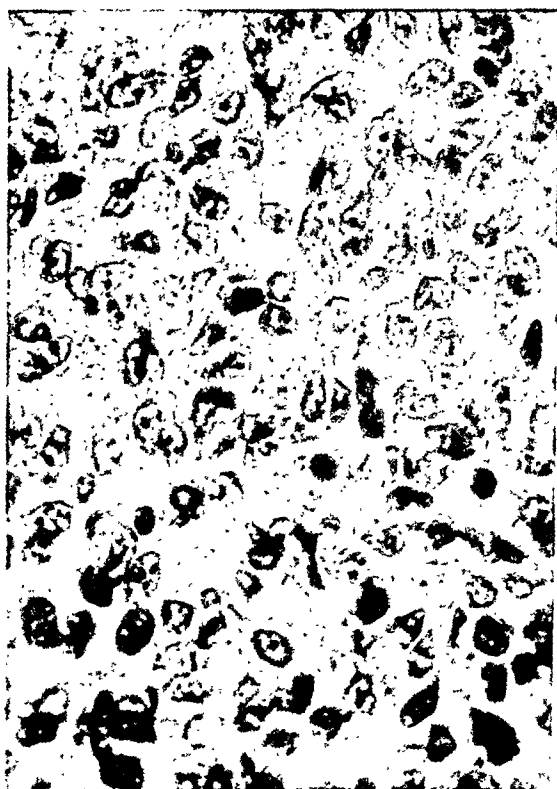


FIG. 7. Slightly reticulated sarcomatous structure. $\times 600$.

kidney, but without invading the renal parenchyma. It was covered by lax, richly vascularized membranes. In section it showed a pale pink, smooth, soft, elastic surface, with small yellow opaque zones, and star-shaped areas of sclerosed tissue, distributed irregularly throughout the tumoral mass. The surface secreted abundant mucus.

Microscopic examination: "Under low power the tissue of the tumor is seen to be continuous, interspersed with numerous cavities of different size and shapes. There are also zones where the cells are more closely packed (Fig. 5). Under high power the continuity of the mesenchymatous and epithelial cells is evident (Fig. 6).



FIG. 5. Low power photomicrograph. In the center a dense zone, surrounded by lax structures. Several cavities are visible. $\times 100$.

The tumoral parenchyma has no precise limits; it forms extensive diffuse zones, or else small rounded ones which occupy a single microscopic field. A typical feature of the tumor is the unequal distribution of the cells; in parts these are closely packed, while in others there is a lax structure with the cells widely spaced. The dense parts occupy the center and the lax ones the periphery of the neoplastic areas; there is no clear cut separation between the two and the latter are apparently transformed into the former in the course of development.

The lax structure is continuous with the connective tissue, and the cells of both intermingle. The perivascular fibrocytes in these areas, as they increase in numbers, become rounded and their nuclei are more loaded with chromatin. Thus cells are formed which still retain the fundamental features of fibroblasts and have some cytoplasmic reticulation (Fig. 7). Gradually, without any definite zone of separation, the lax areas



FIG. 9. Fuchsinophile crystals in a cavity. $\times 700$.

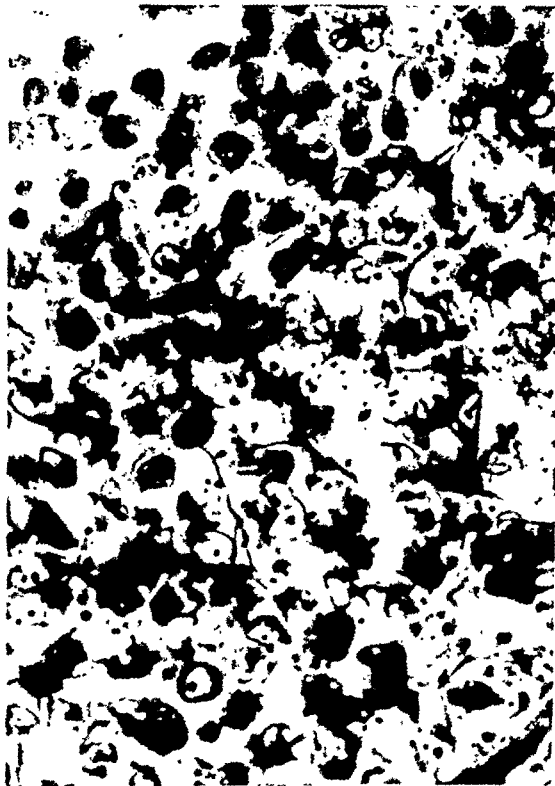


FIG. 10. Spongioplasm of the tumor cells in a dense zone (Río Ortega). $\times 600$.

merge into condensed areas where the cells are separated only by the clefts of sinusoids. The mesenchymatous cells are transformed into the epithelial tumoral cells. Reticulin gradually disappears as the cells take on epithelial characters (Fig. 8).

In other parts the nuclei of the tumoral cells increase in size, the cytoplasm becomes rounded and the cells are separated, appearing as spheric elements with little cytoplasm. In the center of these areas the dissociation of the cells is more marked and cavities are formed (Fig. 6).

The dense parts also show a process of apparent "xanthelasmization" in some of the cells. There are easily recognizable accumulations of polyhedric or spheroid cells with abundant protoplasm, which is finely and evenly vacuolated. The nuclei are frequently

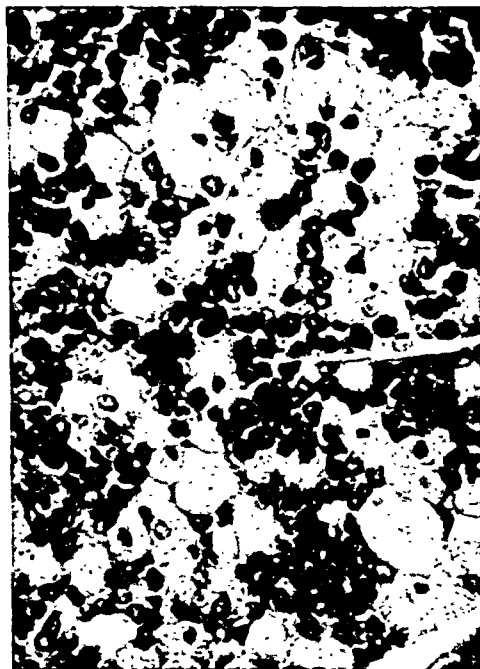


FIG. 8. Transformation of the tumor cells into spongiocytes. $\times 400$.

pyknotic, with no nucleolus, and are sometimes situated in the center and at other times in the periphery of the cell (Fig. 8). These spongiocytes are found usually in the center of the condensed areas. They are in direct contact with a sinusoid or with the lumen of a cavity, forming part of its wall. The size of the spongiocytes varies; sometimes multinucleated cells of large size are found. The vacuolae contain lipids which stain with scarlet red.

Cavities: There are two types of cavities. Some are simple spaces in the tumoral mass without any change of structure in their vicinity; they are of different sizes and numerous enough to give a spongy aspect to the tumor. They are filled with mucus and are apparently due to chemical alterations in the collagenous substance of the stroma. Cavities of the other type are usually larger and of irregular contour. They are found isolated or in groups, and are formed in the dense parenchyma by dissociation of the cells, which

there are sarcomas of corticoadrenal origin. Adami and Wooler (cited by Ewing) described carcinosarcomas due to cellular anaplasia provoked by local factors. We have not been able to consult the originals of these older publications, but more recent ones (1936, 1942) by Cahill and his associates (4, 5), do not report any sarcomatous tumors of the adrenal cortex.

The continuity of the parenchyma and stroma of the tumor here reported

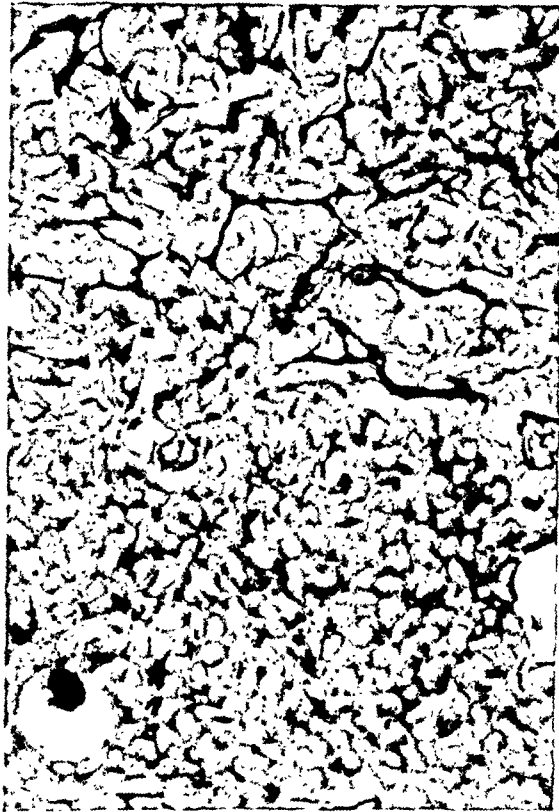


FIG. 12. In the upper half reticulin is evident, in the lower half where the cells are more densely packed there is no reticulin. $\times 400$.

made it appear at first as a sarcoma made up of round and spindle-shaped cells. Further examination of the dense areas and the cavities in their midst showed the epithelial nature of the cells and the gradual transition of the stroma into the epithelial cells. The mesenchymatous origin was thus clearly established. The corticoadrenal nature of these cells was shown by finding what at first seemed a process of "xanthelasmization," but was found to be really the transformation of the cells into spongiocytes loaded with lipids.

The sarcomatous nature of the tumor indicates that its origin should not be looked for in adult corticoadrenal cells, but in an embryonic tissue capable of development and differentiation. The interpretation of the histologic aspect as that of a tumor originating in adult corticoadrenal tissue

form part of the wall of the cavity and sometimes fall into it. The cavity is filled by an albuminoid substance, weakly acidophile. There are also fuchsinophile crystalloid formations within the cavity (Fig. 9).

Spongioplasm: Silver staining for reticulin shows the existence of filaments in the parenchymatous cells, specially developed in the more dense parts and in the cells of the walls of the cavities. These filaments extend into cytoplasmic processes of the cells which are not visible with other stains. This formation is the spongioplasm of the cell which increases as the cell takes on an epithelial aspect (Fig. 10).



FIG. 11. Reticulin in a dense zone (Perdrau). $\times 400$.

Stroma: The stroma of the tumor is formed by the perivascular connective tissue, the reticulin in the dense areas, and numerous foci of sclerosis caused by involution of the parenchyma. The stroma is abundant in the lax areas; it is formed by fine bundles of collagenous fibers and a large number of reticulin fibers. In the denser parts of the tumor there are no collagenous fibers, only a fine network of reticulin (Fig. 11) which disappears in the areas of maximum condensation and epithelization (Fig. 12).

There are ordinary capillaries and sinusoids in the tumoral mass. Hyaline degeneration of the capillary walls occurs, in many places, especially near the sclerotic foci."

DISCUSSION

Ewing (3) refers to three cases of sarcoma originating in the cortico-adrenal epithelium, described by Winkler, but gives no bibliographic reference. The same author quotes Rolleston and Marks' (1898) opinion that

accumulation of glycogen in the liver, has been demonstrated by Seckel (19), and Corey and Britton (20).

We have no data on the glycogen content of the liver in our patient, but perhaps hepatomegaly was caused by an increase in the glycogen content, as is observed in Von Gierke's hepatic glycogenosis.

SUMMARY

A case of a young man suffering from repeated hypoglycemic crises is reported. There was also thyroid hypertrophy, gynecomastia and enlargement of the liver and spleen. A large tumor was found in the right renal fossa and was removed surgically. The hypoglycemic crises did not recur after the operation and the other disturbances rapidly disappeared.

The tumor was classified as a sarcomatous dysembryoplasia with functional differentiation, because cellular elements were found which showed the transformation of embryonic stroma cells into epithelial spongocytes loaded with lipids.

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which suffered anaplastic regression to an embryonic type, cannot be accepted because of the continuity of the stroma and parenchyma, a feature which is not observed in a typical epithelial tissue. Furthermore it must be considered as a tumor of low malignancy, since it did not invade the kidney, nor produce metastases.

From the pathologic point of view the tumor in our patient can be classified as a "sarcomatous dysembryoplasia with corticoadrenal differentiation." Unfortunately it was not possible to study the hormonal content of the tumor, nor to determine the urinary excretion of estrogens which might have enlightened us as to the mechanism of the goiter and gynecomastia observed.

It is interesting to note that gynecomastia has been reported in patients treated with massive doses of insulin (6) and in cases of Addison's disease in which the patients were treated with corticoadrenal extracts, the mammary gland returning to the normal condition when treatment was discontinued (7) (8). Several cases of gynecomastia due to adrenal carcinoma have been reported and Wilkins (9) has recently added another and reviewed the literature.

With respect to the goiter observed, it is useless to speculate on the effect the enlargement of the thyroid may have had on the carbohydrate metabolism in our patient, because there were no symptoms of a disturbance in thyroid function. What effect, if any, the alteration in carbohydrate metabolism may have had on the thyroid, cannot be stated. The only reference in the literature that may be pertinent is the fact observed by Bennett, Koneff and Applegarth (10) that rats with alloxan diabetes have thyroid hypofunction, with significant reduction in the weight of the thyroid. In our patient the hypoglycemic condition may have had the opposite effect, *i.e.*, the enlargement of the thyroid; but we have not found any experimental data on the effect of hypoglycemia on thyroid weight and function to substantiate this (11-16).

However it should be borne in mind that the patient had a definite sign of feminization (gynecomastia); therefore it is possible that the androgen-estrogen equilibrium was altered, and this might have had some influence on the thyroid. Unfortunately there are contradictory data in the literature on this point (17).

Long and his associates (18) have reported that corticoadrenal extracts increased the glycogen stored in the liver, in spite of a rise in blood sugar, in fasting normal and adrenalectomized rats and mice; there was a simultaneous rise in the urinary excretion of nitrogen. Therefore it seems that the adrenal cortex can accelerate the conversion of protein into glycogen. Inhibition of glycogenolysis by corticoadrenal extracts, with subsequent

THE URINARY EXCRETION OF CHORIONIC GONADOTROPIN BY HUMAN FEMALES FOLLOWING PARENTERAL ADMINISTRATION OF AQUEOUS OR BEESWAX SOLUTIONS

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THE primary function of chorionic gonadotropin in the human female would seem to be that of a luteotropic hormone. Parenteral administration of chorionic gonadotropin to normal women results in the increased function of the corpus luteum (1, 2). During the so-called "ovarian phase" of pregnancy, very high levels of this material have been found (3, 4). It is probable that this high level of chorionic gonadotropin stimulates the corpus luteum to secrete amounts of steroid hormones adequate to maintain the pregnancy.

It has been shown that many pregnant women who subsequently abort have low excretion rates of this material (3, 5) and of pregnanediol (6). It would seem that a rational method of treatment of threatened abortion would be to administer adequate amounts of chorionic gonadotropin in order to supplement the deficient placental secretion. This augmentation should stimulate further activity of the corpus luteum.

Since it has been reported that from 10,000 to 20,000 international units of chorionic gonadotropin must be given in a single injection to cause enough material to appear in the urine to produce a positive Aschheim-Zondek test (1), it is probable that large amounts of chorionic gonadotropin must be used to raise the gonadotropin level to the range found in normal pregnancy. This report concerns an attempt to determine the most effective means of maintaining a high level of circulating chorionic gonadotropin.

PROCEDURE

Chorionic gonadotropin (APL),¹ in aqueous solution was given in single intramuscular injections to 8 cyclically menstruating women. The first dose tested was always 5000 I.U. In several patients excretion following different doses was subsequently studied. Five thousand I.U. of the same materi-

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¹ APL supplied through the courtesy of Dr. E. C. Reifenshtein, Jr., of Ayerst, McKenna & Harrison.

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in others the material was present in the first period of collection after injection. The average time before appearance was five hours.

Patient #7, who excreted large amounts of the material following injection of 5000 I.U. on two different occasions, excreted no detectable gonadotropin after injection of 4000 I.U. Patients #1 and #2 excreted no gonado-

Excretion of Gonadotropin following One Intramuscular Injection of Chorionic Gonadotropin

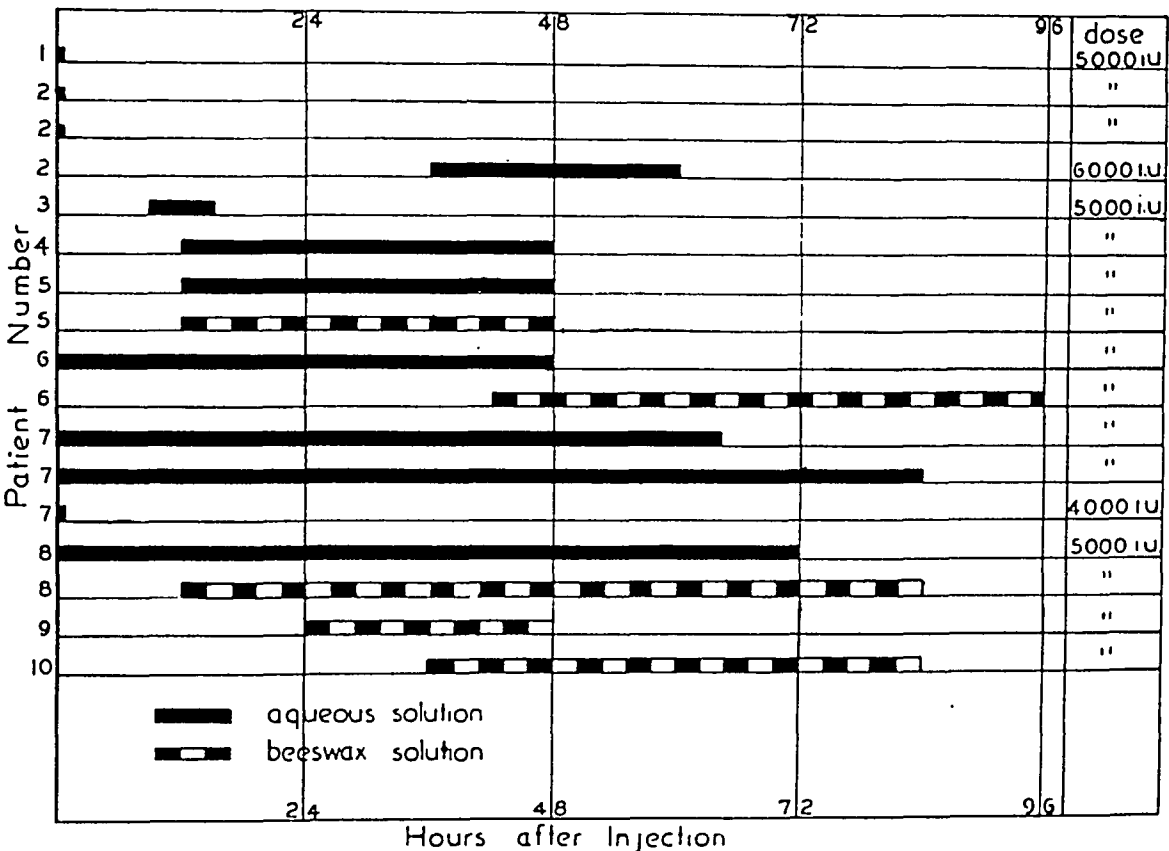


FIG. 1. Each bar represents the period during which chorionic gonadotropin was present in the urine.

tropin after receiving 5000 I.U. Patient #2 was injected at two different times with 5000 I.U. and no gonadotropin could be detected in her urine after either injection. When she received 6000 I.U. she excreted gonadotropin for 24 hours after a 36-hour latent period.

Serum from patients #1 and #4 was tested for gonadotropin. None was detectable at a level of 50 rat units per 100 cc. (2 cc. of serum injected into each of 2 rats), although in patient #4 considerable gonadotropin was present in the unconcentrated urine.

Two menopausal women, one a castrate and the other having a physiologic ovarian failure, excreted no gonadotropin after a single injection of

al dissolved in 1 per cent beeswax was injected at another time to 3 of these patients and to 2 others. The duration of persistence of chorionic gonadotropin in the urine was compared following injection of the gonadotropin in these two different solutions. Excretion of gonadotropin after injection of chorionic gonadotropin in water solution was also studied in 2 postmenopausal women.

A thorough clinical study was made of each patient before injection. All had kept daily basal temperature records and endometrial biopsy specimens had been taken for several months before the administration of the gonadotropin and were repeated following the injection of this material. The endometrial pattern was not altered significantly by the single injection of this material in any of the patients. Neither of the two postmenopausal women had had any evidence of spontaneous ovarian activity for over two years.

Control urine specimens on all patients consisted of three concentrated morning specimens collected before and including the morning of injection. Following the injection, all of the urine was collected in eight-hour or twelve-hour aliquots. Each aliquot was tested for the presence of chorionic gonadotropin until several consecutive specimens had failed to contain detectable amounts of the material. The presence of chorionic gonadotropin in the urine was determined by the hyperemia response of the immature rat ovary. Details of procedures as employed in our laboratory have been published previously (5). One hyperemia unit is equivalent to approximately 3 I.U. of chorionic gonadotropin.

Twenty-four hour excretion of gonadotropin by 6 pregnant women who were threatening to abort was studied before and following injection of chorionic gonadotropin in daily doses of from 5000 to 20,000 I.U. in aqueous solution.

RESULTS

Excretion of injected chorionic gonadotropin by nonpregnant women

The excretion of gonadotropin is expressed schematically in Figure 1. An explanation of that figure is as follows:

Aqueous solution: Ten recovery experiments following a single intramuscular injection of 5000 I.U. of chorionic gonadotropin in aqueous solution were performed using 8 cyclically menstruating women as subjects. Gonadotropin was recovered in the urine of 6 of the 8 patients injected with this dose.

The average period that the material was present in the urine was 39 hours, with a range of from 0 to 80 hours. In some of the subjects, the material did not appear in the urine for twelve hours after injection, whereas

those receiving it in beeswax. The total length of time that it is present in the urine is only slightly longer following administration of the beeswax solution. It is possible that the slower rate of absorption of the beeswax-in-oil solution may result in more effective blood levels of chorionic gonadotropin, but the difficulty of estimating the blood gonadotropin renders it impractical to make direct measurements, so that this point must remain in the realm of conjecture. In our hands, the administration of relatively large amounts of the material in aqueous solution has not been accompanied by more than slight tenderness and no systemic reactions have occurred.

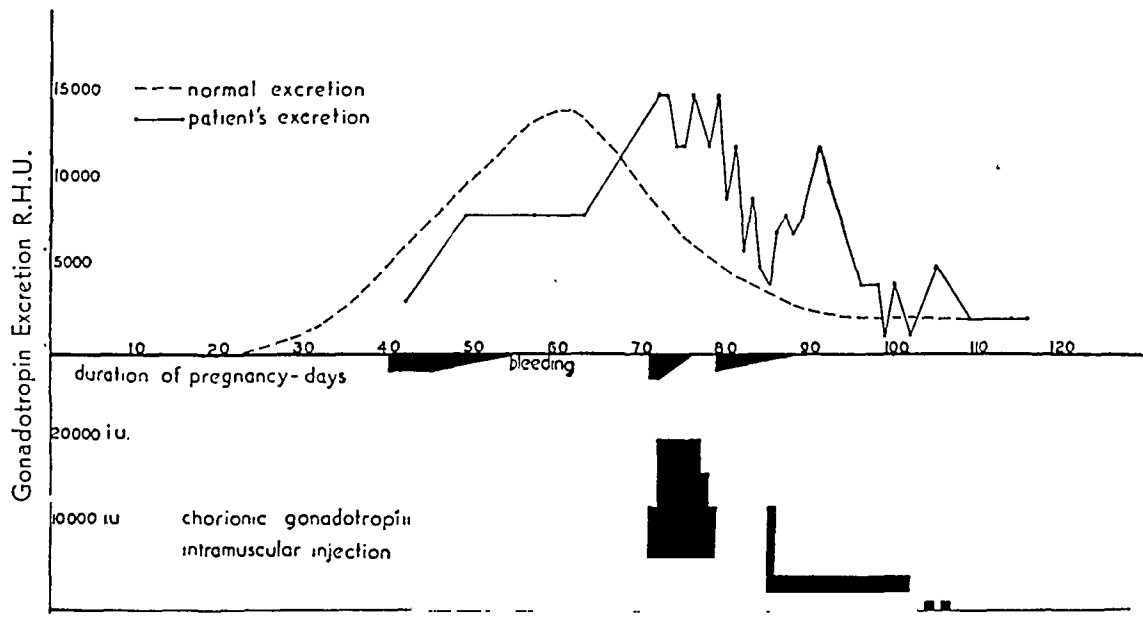


FIG. 2. Excretion of gonadotropin by a pregnant woman with a threatened abortion.

The beeswax-in-oil solution almost always causes considerable local reaction, and in one patient of the five injected, a febrile reaction lasting two days occurred.

The proportion of injected gonadotropin recoverable in the urine is considerable. For example, in one experiment gonadotropin was detectable in the urine of patient #7 for 84 hours. During this time, gonadotropic material equivalent to 530 rat hyperemia units was excreted. A rat hyperemia unit is equivalent to approximately 3 international units. Therefore, gonadotropic activity of over 1500 I.U. was recovered following the injection of 5000 units intramuscularly.

The excretion by the normal female after injection of chorionic gonadotropin of as much, if not more, gonadotropic material than is excreted by patients with inactive ovaries does not support the hypothesis advanced by Jungek, Heller and Nelson (9) that the ovaries normally inactivate gonadotropins. This does not constitute evidence against their hypothesis,

5000 I.U. Each patient then received an injection of 6000 I.U. Only one of them excreted gonadotropin after this injection.

Beeswax solution: Injection of 5000 I.U. of chorionic gonadotropin in beeswax was followed by its appearance in the urine of all 5 patients. There was a latent period in all varying from 12 to 42 hours before the material appeared in the urine. The average latent period was 25 hours. The material was present in the urine for an average of 47 hours, with a range of from 24 to 72 hours.

Each of the 5 patients receiving beeswax complained of local soreness. One patient (#6) developed a fever of 102°F for two days following injection of the beeswax solution.

Plasma vitamin C: Because of the probable importance of vitamin C in steroid hormone synthesis and the change of plasma vitamin C levels in cattle (7) following gonadotropin administration, plasma vitamin C levels² were determined on patients #1 and #4 before and after injection of chorionic gonadotropin. Before injection, average values for patient #1 were 1.26 mg. per cent and for patient #4, 1.3 mg. per cent. These values indicate that there was no deficiency of vitamin C. No change occurred following gonadotropin injection.

Excretion of injected chorionic gonadotropin by pregnant women

Chorionic gonadotropin was administered to 6 women early in pregnancy whose titers of urinary gonadotropin excretion were lower than normal and who were threatening to abort. It was found that the excretion rate could be raised to the normal range when adequate amounts of chorionic gonadotropin were given parenterally. The amount necessary to increase the excretion was usually about 10,000 I.U. daily, although in some patients 5000 I.U. daily caused a definite rise in urinary gonadotropin. Figure 2 shows the typical response of the urinary chorionic gonadotropin of a pregnant woman injected with the aqueous solution. A low excretion rate is raised to the normal range during administration of the material and then again returns to the low range when administration is stopped.

DISCUSSION

The administration of one intramuscular injection of 5000 I.U. of chorionic gonadotropin is followed in most patients by excretion of gonadotropic material in the urine for appreciable lengths of time. Those individuals receiving the material in water begin to excrete it much sooner than

² Determinations of vitamin C were performed at the suggestion of and in the laboratory of Dr. S. J. Tepperman. The method used was that of Roe and Kuether (8).

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since even a vigorous inactivation of gonadotropin by the ovary probably would result in a decrease in amount of gonadotropin too small to be apparent when roughly quantitative techniques such as the method employed in this study are used.

The failure to demonstrate a change in plasma ascorbic acid following gonadotropin administration does not necessarily indicate that none of the vitamin C is utilized by the gonad in steroid synthesis. If this vitamin is employed, the amount used probably is minute compared to the supply available within the body so that no change would be detectable under the conditions of this experiment.

SUMMARY

1. A comparison has been made of the persistence of chorionic gonadotropin in the urine of normal women after injection with aqueous and with beeswax-in-oil solutions of chorionic gonadotropin.

2. A slightly longer duration of excretion of gonadotropic material was detectable following injection of the beeswax solution as compared with the aqueous solution. The beeswax solution injection was followed by a longer interval before appearance of the gonadotropin in the urine, suggesting a slower rate of absorption. Local systemic reactions were frequent following beeswax injection; no reactions followed aqueous solution injection.

3. No change in plasma vitamin C level occurred following injection of chorionic gonadotropin.

4. Excretion of gonadotropin by pregnant women can be increased by parenteral administration of chorionic gonadotropin.³

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³ Since the writing of this paper, Bradbury and Brown (10) have published their findings following injection of aqueous, oil-emulsion, and beeswax preparations of chorionic gonadotropin. Using slightly different methods, they have reached essentially similar conclusions.

and before dinner (group C). A final group of 16 patients was successively treated by diet alone, diet and thyroid medication, and diet and amphetamine medication (group D).

Low calorie diet without any medication (Group A)

This group consisted of 132 patients—18 men and 114 women. The age varied from 13 to 76 years, and the average age for the five weight classes ranged from 45 to 58 years. It was of interest that individuals over 70 were present only in weight classes I and II. The upper age limits in classes III

TABLE 1. TREATMENT WITH DIET ALONE (GROUP A)

Weight class	Over-weight %	Age in years		Sex		Total No. of individuals	Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)
		Range	Average	Male	Female				
I	1-20	31-76	58	9	39	48	11.7	6.2	0.5
II	21-40	22-72	53	5	38	43	10	10.4	1
III	41-60	13-65	47	4	27	31	5.5	7.8	1.2
IV	61-80	26-55	45	0	6	6	5.5	15.04	2.8
V	over 80	38-67	46	0	4	4	5.5	12.25	2.2

to V were 55 to 67 years. Although only comparatively small numbers of individuals were represented in classes IV and V, the general impression was that higher degrees of obesity, as is well known, affected longevity.

The treatment of this group consisted exclusively of a 1200 caloric diet. The comparatively high protein content of the diet (at least 100 Gm.) was stressed previously (1).

The loss of weight in the various weight ranges showed considerable fluctuation. The average monthly loss of weight was 0.5 lb. in class I, which gradually increased to 2.8 lbs. in class IV and decreased slightly to 2.2 lbs. in class V. Despite the slight discrepancy of class V, which included only 4 cases, the impression was that with higher initial overweight there was a higher average monthly loss of weight. A detailed presentation of these data is given in Table 1.

Low calorie diet in combination with thyroid medication (Group B)

This group included 61 patients--2 men and 59 women. The age varied from 15 to 63 years, and the average age for the five weight classes ranged

RESULTS OF PROLONGED MEDICAL TREATMENT OF OBESITY WITH DIET ALONE, DIET AND THYROID PREPARATIONS, AND DIET AND AMPHETAMINE*

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THIS study presents the results of the treatment of obesity under controlled conditions¹ in the Nutrition Clinic of a large general hospital during an observation period of five and one half years. It includes 299 patients—27 men, 272 women. Moderate uncomplicated hypertension without evidence of heart failure was present in about 25 per cent of the group; other forms of cardiovascular disease as well as diabetes were excluded. Each patient remained under regular observation by the same physician at intervals of one to four weeks throughout the entire period of treatment.

The patients were classified according to age, sex and degree of overweight before treatment was initiated. Class I represented those with an overweight of 1 to 20 per cent; class II, 21 to 40 per cent; class III, 41 to 60 per cent; class IV, 61 to 80 per cent; and class V, over 80 per cent. The majority of the patients were in classes II and III. Ideal weight was estimated by the usual standards of sex, age, height and weight from tables of the Life Extension Institute.

Treatment in 132 patients consisted of a low calorie diet of approximately 1200 calories, without any additional medication (group A). In 61 patients, a preliminary period of treatment with diet alone was followed by a second period during which time the same diet was combined with oral thyroid medication, usually 2 to 3 grains of desiccated thyroid daily (group B). In 90 patients a preliminary period of treatment with diet alone was followed by a second period of diet combined with oral administration of 5 to 10 mg. of amphetamine sulphate twice a day one to two hours before lunch

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¹ The patients included in this study were seen in the clinic at regular intervals. Their heart action, pulse rate, blood pressure and body weight were determined regularly. They were given a supply of medicine to cover the interval between visits and on their return, an inquiry was made concerning their adherence to diet and their drug intake. The procedure was, in this respect, the same for groups A, B and C.

3 grains). Special attention was paid to evidences of hyperthyroidism, such as palpitation, tachycardia, and increased perspiration. On the whole, intolerance to therapy was rarely observed. The average weight loss during the diet-plus-thyroid period ranged from 0.4 to 1.6 lbs and thus was certainly less satisfactory than during the diet-alone period. Detailed data for loss of weight in the five classes are presented in Table 2.

The addition of thyroid medication under the conditions of this investigation was of no help in achieving the continued progressive weight reduction in later stages of the treatment.

Low calorie diet in combination with amphetamine medication (Group C)

This group included 90 patients—6 men and 84 women. The age varied from 15 to 64 years and the average age for the weight classes I to V ranged from 34 to 45 years (Table 3). It is evident that the individuals of this group were a few years younger than those of group B and especially those of group A. As is known, hypertension limits the use of amphetamine, and

TABLE 3. PRELIMINARY TREATMENT WITH DIET ALONE AND LATER TREATMENT WITH DIET PLUS AMPHETAMINE (GROUP C).

Weight class	Over-weight %	Age in years		Sex		Total No. of individuals	Diet alone			Diet plus amphetamine		
		Range	Average	Male	Female		Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)	Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)
I	1-20	31-64	45	1	18	19	5.2	1.8	0.3	3.8	4.2	1.1
II	21-40	15-64	42	4	32	36	2.4	4.2	1.7	4.6	8.1	1.8
III	41-60	16-59	36	1	22	23	1.2	1.8	1.5	6.7	10.2	1.7
IV	61-80	25-47	38	0	7	7	2.2	4.2	1.9	2.7	3	1.1
V	over 80	20-40	34	0	5	5	1.9	1.9	1	11.75	19.6	1.7

the observation of this principle restricted the selection of patients in group C to lower age groups.

All patients of group C were treated at first with a 1200 caloric diet for 1.2 to 5.2 months. The average monthly loss of weight during this time varied from 0.3 to 1.9 lbs. Compared with the results in the diet-alone periods in group A and B the response to diet therapy alone in group C was satisfactory.

The preliminary period of diet alone was followed by a second period of 2.7 to 11.8 months during which time the same diet was combined with oral administration of 5 to 10 mg. of amphetamine sulphate twice daily, one to two hours before lunch and before dinner.

from 39 to 47 years (Table 2). Compared with the previous group, this one was composed of somewhat younger individuals. The age difference, which is still more pronounced in group C, is explained by the selection of patients for the various forms of treatment. Individuals of older age groups frequently presented clinical contraindications for the use of thyroid or amphetamine medication and had, therefore, to be excluded from groups B and C.

TABLE 2. PRELIMINARY TREATMENT WITH DIET ALONE AND LATER TREATMENT WITH DIET PLUS THYROID (GROUP B).

Weight class	Over-weight %	Age in years		Sex		Total No. of individuals	Diet alone			Diet plus thyroid		
		Range	Average	Male	Female		Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)	Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)
I	1-20	34-56	47	0	12	12	2.2	3.1	1.3	6.1	2.3	0.4
II	21-40	15-62	43	1	14	15	3.6	4.3	1.2	5	4.4	0.9
III	41-60	19-63	46	0	21	21	3.4	4.4	1.3	5.5	3.5	0.6
IV	61-80	28-50	39	1	8	9	4.6	2.6	0.6	6.7	5.5	0.9
V	over 80	32-57	46	0	4	4	3.6	9.1	2.5	6.3	10.4	1.6

All patients of this group were treated at first by diet alone, approximately 1200 calories, for a period of 2.2 to 4.6 months. The average monthly loss of weight during this time varied from 0.6 to 2.5 lbs. The pattern of weight loss for the various weight classes, however, is different from that in group A. The average monthly weight loss in group B was almost identical for the weight classes I, II, III (1.2 to 1.3 lbs), decreased considerably in class IV (to 0.6 lb) and rose to the peak of 2.5 lbs. in class V. Because of the small number of individuals included in weight classes IV and V no definite significance can be attached to the results in the latter two classes. Individuals of weight classes I to III of group B showed a better response to diet alone than those in group A. The absence from group B of very old individuals (whose cooperation as a whole was less satisfactory than that of younger individuals) may offer an explanation for this observation. It was frequently noted that men and women over 60 had resigned themselves to their obesity and showed lack of cooperation and indifference.

The preliminary period of diet alone was followed by a second period of 5 to 6.7 months during which time the same diet was combined with oral administration of 1 to 4 grains of desiccated thyroid daily (usually 2 to

decreased later to 0.3 to 0.4 lb. Finally, in group C, the average monthly weight loss during the initial period was 3 to 3.3 lbs and dropped later to 0.7 to 2.1 lbs. Only weight classes I, II and III were used for this analysis since the majority of the individuals were included in these three weight classes.

TABLE 4. PRELIMINARY TREATMENT WITH DIET ALONE AND LATER TREATMENT WITH DIET PLUS THYROID OR DIET PLUS AMPHETAMINE.

	Group A			Group B			Group C		
	Diet alone			Diet plus thyroid			Diet plus amphetamine		
Overweight classes	I 1-20 %	II 21-40 %	III 41-60 %	I 1-20 %	II 21-40 %	III 41-60 %	I 1-20 %	II 21-40 %	III 41-60 %
Average monthly weight loss in pounds during the 1st to 2nd months	2.5(9)*	3.84(11)	3.32(13)	2.62(2)	3.30(7)	1.52(9)	3.02(9)	3.0(14)	3.3(8)
Average monthly weight loss in pounds during the 4th to 20th months	1.0(22)	2.15(26)	1.8(17)	0.30(7)	0.35(6)	0.43(9)	0.68(6)	2.1(15)	1.55(10)

* The figures in parentheses represent the number of individuals.

It is thus evident that the 'time factor' played an important role in all three weight groups. The initial results were far superior to those obtained later. Diet alone compares favorably with diet and amphetamine, whereas diet and thyroid was disappointing in the later stages of treatment. It is therefore most important in studies of this type to separate the short-term effect strictly from the long-range results.

Untoward effects of thyroid and amphetamine medication

The pharmacology and toxicology of reducing drugs such as thyroid and amphetamine is widely known (2, 3, 4, 5, 6). In our own experience thyroid medication resulted only occasionally in tachycardia or palpitation. These symptoms were easily controlled by reduction of the dose or by discontinuation of medication.

In the use of amphetamine sulphate strict medical indications were observed. Nevertheless, occasionally the known side effects such as ir-

The average monthly loss of weight during the amphetamine period ranged from 1.1 to 1.8 lbs. The results are better than those achieved under similar circumstances with thyroid medication in group B. Thus, the continuation of a satisfactory reducing regimen over longer periods gave better results with amphetamine than with thyroid medication. However, a certain caution is indicated because of the different rate of weight reduction observed in the preliminary diet-alone periods in groups B and C.

Diet alone, diet and thyroid medication and diet and amphetamine medication used successively in the same individual (Group D)

This group included 16 individuals whose age ranged from 20 to 65 years. Four of them were in weight class I, 7 in weight class II, 2 in weight class III, and 3 in weight class IV. All patients were treated first by diet alone over a period of 0.75 to 10 months (average 3.8 months). In the second period desiccated thyroid was added and in the third period, amphetamine, the same quantity as used before in groups B and C. The sequence varied with the individuals, as some were first subjected to amphetamine and then to thyroid medication. The thyroid medication was administered over a period of 0.5 to 27 months (average 6.8 months) and the amphetamine medication over a period of 0.5 to 15 months (average 3.3 months). Again there were wide fluctuations, but the average figures for the four weight classes confirmed the results obtained with larger numbers of individuals in groups A to C. The average loss of weight in the diet-alone period in this group was 0.6 lb.; with the addition of thyroid, 0.1 lb.; and with the addition of amphetamine, 0.9 lb. Thus in the prolonged treatment of obesity, diet *per se* was more effective than diet plus thyroid and somewhat less effective than diet plus amphetamine.

The 'time factor' in weight reduction

Best results were obtained in the first one to two months of treatment regardless of the form of therapy used. This fact was primarily due to the greater degree of overweight at the beginning of treatment. In our opinion an additional factor was the morale of the patient which was at its best during the first one to two months of treatment. In later phases of treatment less cooperation was encountered.

It was of interest to compare the initial results obtained with diet alone, diet and thyroid, and diet and amphetamine medication with the later results of the same groups and weight classes (Table 4). In group A the average monthly weight loss during the initial period of one to two months was 2.5 to 3.8 lbs. while in the later course of four to twenty months the corresponding figures were 1 to 2.15 lbs. The results were even more striking in group B where the initial weight loss varied from 1.5 to 3.3 lbs. and

With sedatives and large amounts of fluids the symptoms of intoxication subsided in the course of twenty-four hours.

In our opinion, amphetamine preparations should not be dispensed and refilled without a physician's prescription. These drugs should not be used routinely in the treatment of obesity. Patients to whom they are administered should be closely observed (18).

The discontinuation of medication confronts many patients with rather difficult psychologic problems. Obese individuals are as a rule inclined to believe, erroneously, that overweight and fatness are caused by factors beyond their control. Even unusually intelligent and critical individuals are rarely willing to realize and/or admit the importance of overeating in their own case of fatness. A smaller group of obese individuals is fully aware of the latter factor but lack the energy and the will power necessary to adhere to restricted diets and radically to change their eating habits. A drug used in the treatment of obesity assumes the role of a fetish protecting the "unfortunate" individual against the effects of overeating. Its prolonged use, however, is loaded with psychologic hazards because it confirms the first type of patient in his false ideas, or it supplies the second with an "ersatz" morale of limited transitory value.

The discontinuation then in both types of individuals causes a vacuum and leaves them suddenly without these protective mechanisms. Increased frustration and depression often result and add a new link to the vicious circle: frustration—overeating—increased frustration—more overeating.

Observations of this sort should make a physician more than reluctant in the use of any "reducing" drug in obesity for protracted periods. Because of the peculiar psychosomatic set-up, these drugs used as fetishes against overeating prevent the average obese patient from the full realization that permanently satisfactory results can be achieved only by reducing food intake and by no other means.

SUMMARY

A group of 299 obese patients treated under controlled conditions in the Nutrition Clinic of a large general hospital showed wide fluctuations in the therapeutic results. In group A, in which only low calorie diets were employed, the average weight loss increased in proportion with the degree of obesity. Diet alone resulted in better weight reduction than diet plus thyroid medication (group B), whereas diet plus amphetamine (group C) showed somewhat better results than diet alone. In the latter group there were striking appetite-reducing effects of amphetamine sulphate with considerable loss of weight. The initial effect often faded under continued administration of the drug so that with the same dosage of amphetamine, increased food intake and weight gain resulted; to avoid this, in many in-

ritability, nervous tension, palpitation, headaches, insomnia and constipation compelled us to use smaller doses or to discontinue the drug.

A rather frequent complaint was dry mouth and often in association with it, halitosis. The impression was that the inhibition of salivation affected the bacterial flora of the oral cavity and thus resulted in an unpleasant, sweetish odor which was noticed by the patients and their families. One of our patients refused the drug on Saturdays and Sundays when she had social dates and dances from fear that her breath might be offensive. Other patients used chewing gum, "Sen Sen," and/or cigarettes to camouflage the unpleasant breath. It was advisable to stop medication during upper respiratory infections in order not to enhance the sensation of dryness of the mucous membranes. Better results were encountered with amphetamine in spring, fall and winter than during the hot summer months. A few of our female patients preferred not to use the drug premenstrually. In their observation the sensation of heaviness and congestion in the lower abdomen was enhanced by its use.

Much has been written recently on the etiology of obesity (7) with special emphasis on psychologic factors (8, 9, 10). The compulsive appetite is considered to be an expression of disturbed emotional life and oral gratification derived from eating represents compensation for frustration and "emotional starvation." Insecurity, professional disappointment and personality defects can be easily compensated by excessive eating. Amphetamine and its derivatives exert their effect not only by suppression of appetite but also by a special action upon the central nervous system (11).

The stimulating effects of amphetamine are noted by the patients, who occasionally will increase the dose of the drug to amounts detrimental to their health, without consulting the physician. This is done because increasingly larger doses are needed for the maintenance of the desired effects on mood and feeling of well-being. Although toxicity of amphetamine has been claimed to be minimal, poisoning by accident or uncritical use has been reported (12, 13, 14, 15, 16, 17). In our own observation two instances of amphetamine poisoning were observed. They concerned two psychoneurotic women from the private practice of one of us (D.A.) and were not included in this study. These patients refilled the prescriptions for amphetamine sulphate on many occasions without our knowledge and gradually, because of depression, raised the dose to 100 mg. per day. In both instances the results were extreme irritability and restlessness, insomnia, tachycardia and rapid respiration. The diagnosis of amphetamine poisoning was later confirmed when we learned of the patient's admission of the abuse of the drug. In both instances acute exacerbations of deeply rooted psychologic conflicts might have been additional precipitating factors for the use of toxic doses. No serious damage was encountered.

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stances the dose of amphetamine had to be gradually raised. This is in agreement with observations from this hospital in the treatment of diabetes associated with obesity (19).

Regardless of the form of therapy employed, best results were obtained in the first one or two months of treatment. In the later stages of treatment amphetamine proved to be superior to thyroid. Nevertheless, the long-term results with diet alone compared favorably with those obtained with diet and amphetamine. It is imperative in studies on weight reduction in obesity to separate strictly the short-term effect from the long-range results. The immediate success is usually much better than the ultimate result.

The untoward effects of thyroid and amphetamine medication have been discussed. Some individuals developed addiction to amphetamine and a warning against the indiscriminate use of the drug is in order. Two instances of amphetamine poisoning were observed in psychoneurotic individuals who refilled the prescription for amphetamine sulphate on many occasions and raised the dose to toxic levels. Although no serious complications resulted in these cases, amphetamine may cause poisoning by accident or by uncritical use.

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the oscillations are due to contractions of the tubes, as they are obtainable in patients who have undergone bilateral salpingectomy, as long as there exists an outlet for the gas, however small, such as that remaining after puncture of the uterine wall with a needle. Stabile considers the oscillations

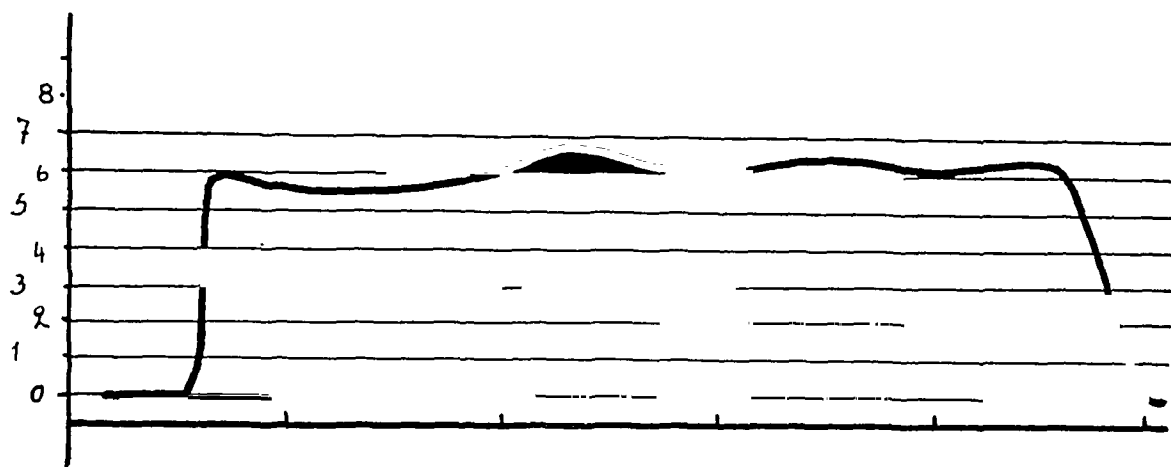


FIG. 1. Utero-tubal persufflation curve (cm.) in Case 1 before treatment.



FIG. 2. Utero-tubal persufflation curve (cm.) in Case 1 after patient was treated with desiccated thyroid.

to be due to variations in the resistance of the uterus and tubes to the passage of a gas, and supposes this resistance to be directly related to the tone of the uterine and tubal muscle. Caviglia (10) confirmed Stabile's investigations. Whichever explanation be admitted—contractions of the tubes (Rubin) or variations in the tone of the uterus and tubes (Stabile)—the oscillations are evidently of muscular origin.

In this report we are publishing the kymographic persufflation curves obtained during the postmenstrual period in 7 myxedematous women before and after treatment with desiccated thyroid.

¹ The desiccated thyroid was prepared by Di-pert Laboratories, Montevideo; iodine content, 0.30 Gm. per cent.

UTERO-TUBAL PERSUFFLATION CURVE IN MYXEDEMA. EFFECT OF THYROID THERAPY

J. C. MUSSIO FOURNIER, M.D.* AND A. POU DE SANTIAGO, M.D.

Institute of Endocrinology, Montevideo, Uruguay

IN myxedematous patients, anatomic and functional changes take place in various organs containing muscular tissue. Thus, to mention but a few of the published observations, dilatation of the heart was described by Zondek (1) in 1918, megacolon by Schippers (2) in 1929, dolicoecolon by Crismer (3) in 1947, megaduodenum by Hillemand and Dugué (4) in 1947, atony of the bladder by Evans (5) in 1932. Constipation is a common finding. All these disorders disappear with the administration of desiccated thyroid.

These facts led us to investigate the condition of the muscles of the uterus and fallopian tubes in patients with myxedema. For this purpose we used persufflation of the uterus, following the utero-tubal kymographic persufflation technic as described by Rubin (6) in 1928. Our apparatus—the usual application of which is in the study of the patency of the uterine tubes—is similar to that of Rubin. It consists of a gas container from which CO₂ is supplied to the uterine cavity at the constant rate of 60 cc. per minute. The gas flows through a special cannula with an encircling rubber cone which, when pressed into the external os, ensures a gas-tight joint. A manometer is connected on a branch from the piping which carries the CO₂ to the uterus. The manometer is a U-shaped tube containing mercury and provided with a scale and a float, the movements of which are registered on a chart fixed to a drum which revolves at a peripheral rate of 4 cm. per minute. It should be borne in mind that with this manometer, a height of 1 cm. on the graph is equivalent to a CO₂ pressure of 2 cm. of mercury.

After persufflation of the uterus and fallopian tubes with CO₂, a curve is obtained showing oscillations, provided that at least one of the tubes be permeable. Three elements are to be studied in these curves, *viz.*, the height of the curve and the frequency and amplitude of oscillations. In the woman of childbearing age, the height of the curve is 6 or 7 cm., the frequency of the oscillations is 4 or 5 per minute and their amplitude, 10 to 12 mm., when obtained with the usual consumption of gas as given by Rubin. In ovarian insufficiency all three elements were found to be diminished or even absent (Rubin (7)). Rubin considered the oscillations to be due to contractions of the uterine tubes. Stabile (8, 9) does not believe that

* Director of the Institute.

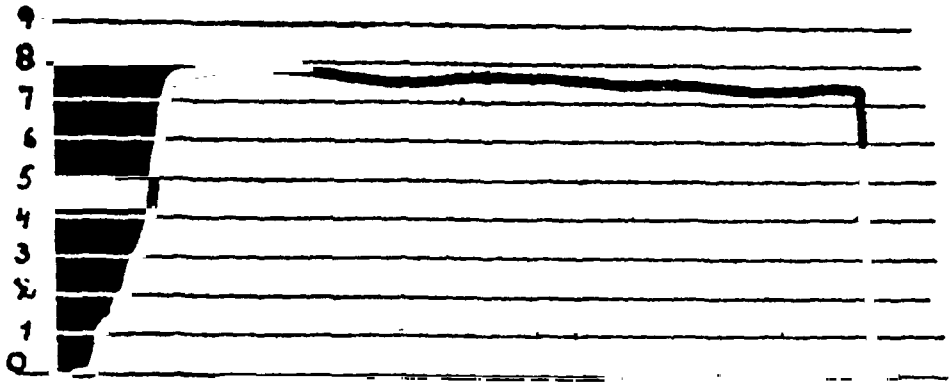


FIG. 5. Utero-tubal persufflation curve (cm.) in Case 4 before treatment.



FIG. 6. Utero-tubal persufflation curve (cm.) in Case 4 after patient was treated with desiccated thyroid.

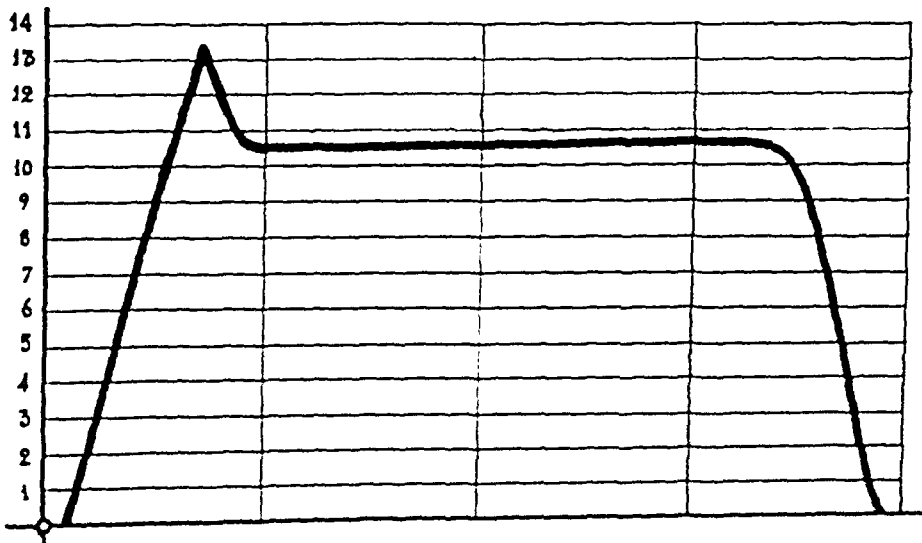


FIG. 7. Utero-tubal persufflation curve (cm.) in Case 5 before treatment.

Case 1. Typical myxedema of two years' duration in a woman 45 years of age. Menstruation was regular in a 3/30 day cycle. The basal metabolic rate (B.M.R.) was minus 13 per cent. The curve shown in Figure 1 is 6 cm. high, which is below normal, and it has very few oscillations. Desiccated thyroid¹ in a daily dose of 0.05 Gm. (3/4 grain) was then given for four months. The B.M.R. rose to plus 4 per cent. A second curve was obtained (Fig. 2). The height of the curve had increased and oscillations of great amplitude and of normal frequency were observed.

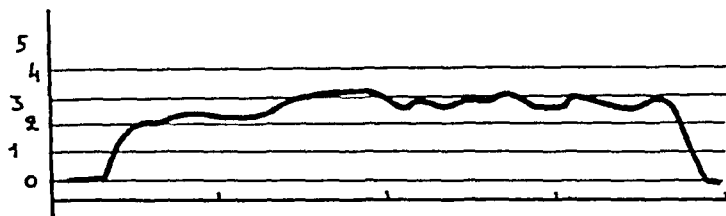


FIG. 3. Utero-tubal persufflation curve (cm.) in Case 2 before treatment.

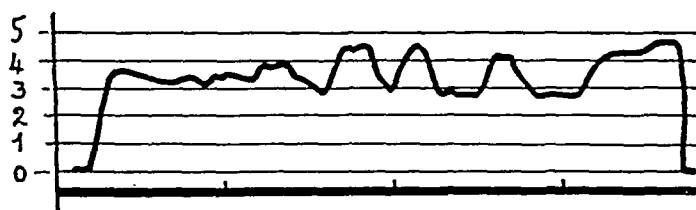


FIG. 4. Utero-tubal persufflation curve (cm.) in Case 2 after patient was treated with desiccated thyroid.

Case 2. Myxedema of two years' duration in a woman 37 years of age. Menstruation was regular. The B.M.R. was minus 26 per cent. The persufflation curve (Fig. 3) was low (between 3 and 2 cm.) but the oscillations were normal. The patient was treated with 0.10 Gm. (1½ grains) of desiccated thyroid daily for six months, with clinical improvement. The B.M.R. rose to minus 1 per cent. A new curve (Fig. 4) showed increased height, and oscillations that were normal in both frequency and amplitude.

Case 3. Typical congenital myxedema in a woman 25 years of age. Height 107 cm. Menstruation was regular in a 2/34 day cycle. The B.M.R. could not be obtained. A series of endometrial biopsies showed a complete menstrual cycle with proliferative and secretory changes. The persufflation curve was low (3 to 5 cm.) with a few oscillations of small amplitude.

Case 4. A woman 33 years of age with myxedema which began in 1935 after her fifth pregnancy. Except for amenorrhea of two months' duration, menstruation was regular in a 3/20 day cycle. The genital tract was organically normal. She showed complete alopecia, and her B.M.R. was minus 29 per cent. The persufflation curve (Fig. 5) was 7 to 8 cm. in height with no oscillations. After one month of treatment with 0.10 Gm. (1½ grains) daily of desiccated thyroid, the patient was greatly improved; the myxedema disappeared, the hair began to grow and the B.M.R. rose to minus 9 per cent. The persufflation curve was 9 to 12 cm. in height, with a few oscillations. After two years of

menstruated regularly in a 6/30 day cycle. The persufflation curve was normal in height but showed almost no oscillations. After five months of treatment with 0.10 Gm. ($1\frac{1}{2}$ grains) of desiccated thyroid daily the B.M.R. rose to minus 7 per cent. The persufflation curve showed the same height but normal oscillations.

Case 7. A woman 42 years of age. Myxedema began twenty years previously, four years after the birth of her only child. Her genital tract was organically normal and she menstruated regularly in a 7/30 day cycle. Her B.M.R. was minus 28 per cent. Figure 9 shows a persufflation curve of normal height but without oscillations. After three months of treatment with 0.05 Gm. ($\frac{3}{4}$ grain) daily of desiccated thyroid there was a general improvement. The B.M.R. rose to minus 5 per cent. The persufflation curve (Fig. 10) increased in height and showed oscillations of normal amplitude and frequency.

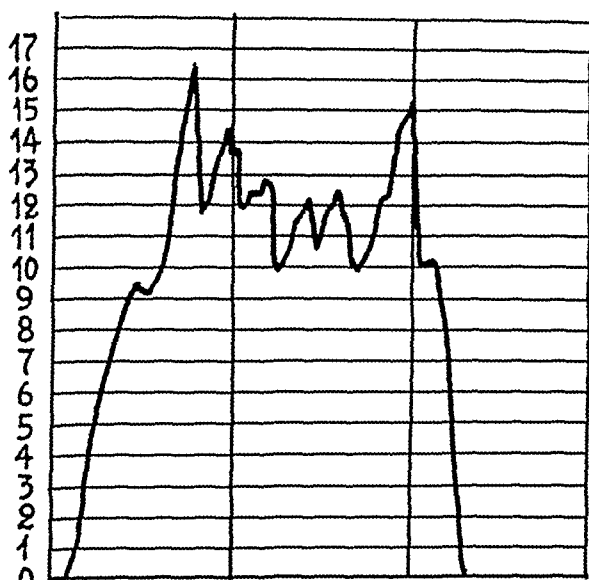


FIG. 10. Utero-tubal persufflation curve (cm.) in Case 7 after patient was treated with desiccated thyroid.

SUMMARY

A study illustrated with seven case reports is presented.

It was found that persufflation curves of the uterus and fallopian tubes in myxedematous women may be diminished in height or have oscillations which are of smaller frequency and amplitude than those of normal subjects.

Thyroid therapy produces normalization of the utero-tubal persufflation curve, coincidentally with clinical improvement and increased basal metabolism.

Whatever mechanism be accepted as explaining the elements of the kymographic curve, it is evident that a muscular factor intervenes in its pathologic modifications. This is not surprising in view of the changes which have been observed in the muscles of the heart, intestines and bladder of myxedematous patients.

thyroid treatment the patient was almost normal; her B.M.R. was plus 4 per cent and there were no symptoms of myxedema and no alopecia. The persufflation curve was of normal height (9 to 11 cm.) with oscillations which were normal in amplitude and frequency (Fig. 6).

Case 5. A woman, 44 years of age, in whom the diagnosis was infantile myxedema. Height 120 cm. Her voice was hoarse. Menstruation occurred in a 7/26-30 day cycle. Her B.M.R. was minus 19 per cent. A persufflation curve (Fig. 7) was normal in height (10 to 11 cm.) but showed no oscillations. Thyroid treatment for nine months with

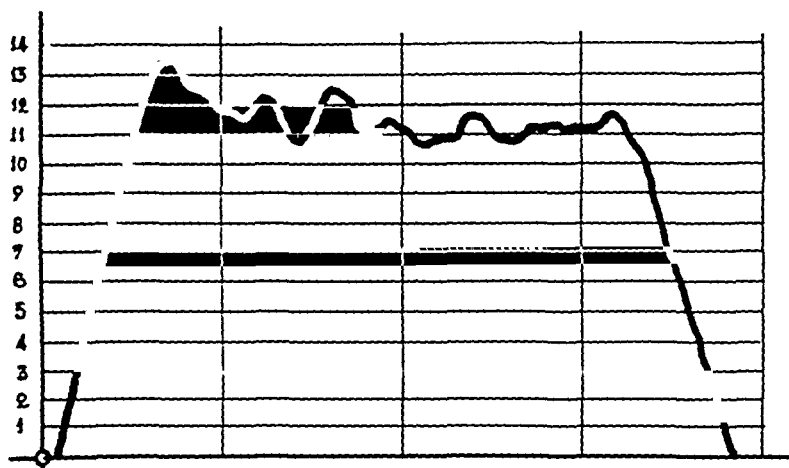


FIG. 8. Utero-tubal persufflation curve (cm.) in Case 5 after patient was treated with desiccated thyroid.

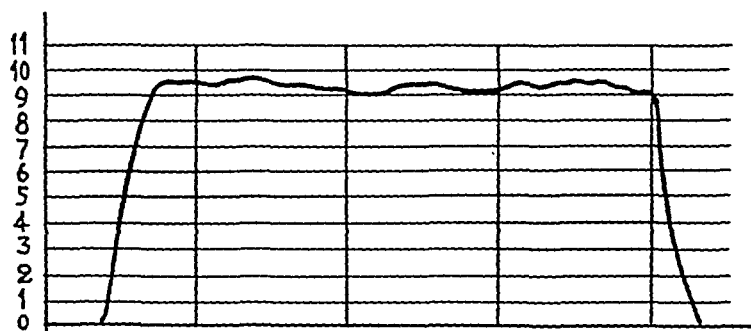


FIG. 9. Utero-tubal persufflation curve (cm.) in Case 7 before treatment.

0.05 Gm. (3/4 grain) of desiccated thyroid daily resulted in great physical and psychic improvement. The B.M.R. rose to minus 6 per cent. The persufflation curve was higher than before (11 to 13 cm.) and it showed oscillations of subnormal frequency and amplitude (Fig. 8).

Case 6. Hypothyroidism of many years' standing in a woman 32 years of age. There was an anemia with a red cell count of 2,400,000 and hemoglobin 55 per cent. The B.M.R. was minus 23 per cent. She had had four pregnancies, had normal genital organs, and

The 1949 Meeting of the Association for the Study of Internal Secretions

The Thirty-First Annual Meeting of the Association for the Study of Internal Secretions will be held in the Chalfonte-Haddon Hall, Friday and Saturday, June 3 and 4, 1949, in Atlantic City, New Jersey.

We are informed by the hotel management that reservations will be difficult to secure on short notice; therefore, members are urged to make reservations at once with Chalfonte-Haddon Hall, giving time of arrival and length of stay in Atlantic City.

The scientific sessions will be held in the Viking Room, as formerly, and registration will be on the same floor. The annual dinner will be held in the Rutland Room, Friday, June 3, at 7 p.m., preceded by cocktails in the same room.

Those wishing to present papers, which will be limited to ten minutes, should send the title and four copies of an abstract of not more than 200 words, to Dr. J. S. L. Browne, Royal Victoria Hospital, Montreal 2, Canada, not later than March 1, 1949. It is imperative that the abstracts be informative and complete, with results and conclusions, in order that they may be of value for reference and suitable for printing in the program.

Nominations for the Squibb and Ciba Awards and the Ayerst, McKenna and Harrison Fellowship should be filed on special forms with the Secretary of the Association, not later than March 15, 1949, according to specifications given in the section on Awards.

Announcement of Jefferson Medical College and Hospital Fellowship

A Fellowship in obstetric and gynecologic endocrinology will be available at the Jefferson Medical College and Hospital, Philadelphia, on or about May 1, 1949, under the direction of Dr. A. E. Rakoff, Assistant Professor of Obstetrics and Gynecology, and Endocrinologist to the Department of Clinical Laboratories.

The Fellowship is available to Doctors of Medicine who have had at least one year or its equivalent of postgraduate training in obstetrics and gynecology. Applicants for the Fellowship should communicate at once with Dr. Lewis C. Scheffey, Professor of Obstetrics and Gynecology, Head of Department, and Director of Division of Gynecology, Jefferson Medical College and Hospital, Philadelphia 7, Pa.

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Antithyroid Drugs. (Round Table Discussion)

E. B. Astwood, M.D., Boston, Massachusetts

Radioactive Iodine. (Round Table Discussion)

Mayo Soley, M.D., Iowa City, Iowa

Current Treatment of Hyperthyroidism. (Round Table Discussion)

John de J. Pemberton, M.D., Rochester, Minnesota

Treatment of Hyperthyroidism. (Round Table Discussion)

J. H. Means, M.D., Boston, Massachusetts

When is a Malignant Goiter Malignant?

Robertson Ward, M.D., San Francisco, California

Incidence of Carcinoma of the Thyroid in Nodular Goiter.

Warren Cole, M.D., Chicago, Illinois

What Thyroid Nodules Are to be Feared? A Basis for Deciding upon Surgical Exploration.

Oliver Cope, M.D., Boston, Massachusetts

Papillary Tumors of the Thyroid.

Shields Warren, M.D., Boston, Massachusetts

Non-encapsulated Sclerosing Tumor of the Thyroid.

J. Beach Hazard, M.D., and George Crile, Jr., M.D., Cleveland, Ohio

The Natural History of Thyroid Cancer.

Edgar L. Frazell, M.D., and Frank W. Foote, Jr., M.D., New York, New York

Lymphosarcoma of the Thyroid

Robert S. Dinsmore, M.D., Cleveland, Ohio

Surgical Treatment of Carcinoma of the Thyroid.

B. Marden Black, M.D., Rochester, Minnesota

Radio-Iodine Therapy of Metastatic Carcinoma of the Thyroid: A Six Year Progress Report.

S. M. Seidlin, M.D., Miss E. Oshry, I. Rossman and E. Siegel, New York, New York

Radio-Iodine in the Treatment of Metastatic Cancer of the Thyroid—Credits and Debits.

Jack B. Trunnell, M.D., Miss Ruth Hill, Benedict J. Duffy, Jr., M.D., Leonidas Marinelli, M.D., Wendell Peacock and Rulon W. Rawson, New York, New York

Presidential Address—"Henry S. Plummer"

Arnold S. Jackson, M.D., Madison, Wisconsin

The Problem of Cancer of the Thyroid in a Non-Endemic Goiter Area

David H. Poer, M.D., Atlanta, Georgia

Some Goiter Problems Met With at West China Union University, Chengtu, Szechwan, China.

Charles H. Arnold, M.D., Lincoln, Nebraska

Cretinism.

Harry Colfer, M.D., Madison, Wisconsin

New Discoveries on the Innervation of the Larynx.

Brien T. King, M.D., and Ralph Gregg, M.D., Seattle, Washington

Metabolism Testing Under Anesthesia in Normal and Hyperthyroid Subjects.

Elmer C. Bartels, M.D., Boston, Massachusetts

Relationship of Lymphocytes and Fibrous Replacement to the Incidence of Postoperative Myxedema.

F. B. Whitesell, Jr., M.D., and B. Marden Black, M.D., Rochester, Minnesota

Struma Lymphomatosa.

T. C. Davison, M.D., and A. H. Letton, M.D., Atlanta, Georgia

The 1949 Meeting of the American Goiter Association

The annual meeting of the American Goiter Association will be held at the Loraine Hotel in Madison, Wisconsin, May 26 to 28, 1949. All members who have not made their hotel reservations are urged to do so immediately.

PRELIMINARY PROGRAM

Dietary Factors in the Pathogenesis of Simple Goiter.

Monte A. Greer, M.D., Martin G. Ettlinger, M.D., and E. B. Astwood, M.D.,
Boston, Massachusetts

Comparative Activity of Thiouracil and Other Antithyroid Compounds in the Rhesus Monkey.

D. A. McGinty, M.D., and M. L. Wilson, M.D., Detroit, Michigan

The Metabolic Fate of the Thyroid Hormone or its Derivatives.

W. T. Salter, M.D., New Haven, Connecticut

Metabolic Studies with I^{131} Labelled Thyroid Compounds.

Alexander Albert, M.D., and F. Raymond Keating, M.D., Rochester, Minnesota

The Calorigenic Properties of Tetrabromthyronine Tetrachlorthyronine as Assayed in Human Myxedema.

Jacob Lerman, M.D., Boston, Massachusetts

Thyroid Hormone-Like Properties of Tetrabromthyronine and Tetrachlorthyronine.

Charles E. Richards, M.D., Roscoe O. Brady, M.D. and Douglas S. Riggs, M.D.,
Boston, Massachusetts

The Antithyroxin Activity of Thyroxin Analogues.

Ruth Cortell, M.D., New York, New York

Thyroid-thyrotrophic Hormone Interaction in Body Fluids as Tested in the Starved Tadpole.

Savino A. D'Angelo, M.D., New York, New York

The Van Meter Prize Award Paper.

To be presented by winner of the Award

The Confessions of an Elderly Thyroidologist.

J. H. Means, M.D., Boston, Massachusetts

The Effects of Massive Doses of Potassium Iodide.

T. S. Danowski, M.D., Pittsburgh, Pennsylvania

The Thyroxin-like Action of Elemental Iodine

Samuel Dvoskin, M.D., New York, New York

The Functional Capacity of Various Types of Thyroid Carcinoma as Revealed by the Autoradiographic Demonstration of Radioactive Iodine.

Patrick J. Fitzgerald, M.D., New York, New York

A Method for the Preoperative Estimation of Function of Thyroid Tumors: Its Significance in Diagnosis and Treatment.

Brown M. Dobyns, M.D., and Bengt N. Skanse, M.D., Boston, Massachusetts

Thyroidectomy. (Round Table Discussion)

Richard B. Cattell, M.D., Boston, Massachusetts

comparative anatomy, and the fine arts, to comprise at least ninety semester hours in a college of arts and sciences approved by the Council on Medical Education and Hospitals of the American Medical Association, and/or in a School of Fine Art approved by the Educational Division of the Veterans Administration. The School of Medicine reserves the right to require more than the minimum hours here set down for admission. Tuition and fees will be charged in conformity with tuition and fees for the School of Medicine of the University of Georgia. The course consists of the following:

- (a) Surface anatomy.
- (b) Anatomical dissection and drawing.
- (c) The study of fresh and hardened specimens.
- (d) Microanatomy and its techniques.
- (e) Lettering and presentation.
- (f) Design and preparation of charts and schemata.
- (g) Surgical procedures and reconstructions.
- (h) Techniques of medical illustration.
- (i) Mechanics and ethics of medical publishing.
- (j) Format and design of monographs and books.
- (k) History of medical illustration.
- (l) Medico-visual education and direction.

FIRST TRIMESTER

Student Registration—5 September, 1949

Jack Wilson, Associate Professor of Art as Applied to Medicine, and Director of Illustration.

Mary P. Hallinan, Student Assistant for Art as Applied to Medicine.

The 1949 Annual Meeting of the American Diabetes Association

CHALFONTE-HADDON HALL,
ATLANTIC CITY, N. J.

SATURDAY AFTERNOON, JUNE 4;
SUNDAY MORNING AND AFTERNOON, JUNE 5.

BANQUET, SATURDAY NIGHT.

Please send reservations for the banquet now to this office. Wives of members are welcome. Dinner subscription—\$6.00—*Payable when you register at the meeting.*

Total Thyroidectomy: A Supplemental Report of 280 Cases of Diffuse Toxic Goiter Treated by this Method.

A. C. Scott, Jr., M.D., and P. M. Ramey, M.D., Temple, Texas

Pneumothorax Following Thyroidectomy (A Report of Two Cases)

Lindon Seed, M.D., Chicago, Illinois

A Simplified Clinical Method for the Determination of Blood Iodine.

Arthur C. Connor, M.D., Roy E. Swenson, M.D., George M. Curtis, M.D., Columbus, Ohio

Treatment of Recurrent Hyperthyroidism.

William S. Reveno, M.D., Detroit, Michigan

COURSES IN MEDICAL ILLUSTRATION UNIVERSITY OF GEORGIA SCHOOL OF MEDICINE

The first school of medical illustration in the southeastern United States has been opened at the University of Georgia School of Medicine.

The courses are designed to equip illustrators for all types of scientific illustration, as shown in Paragraphs B1 and B2 of the following announcement. Since a knowledge of the techniques of medical illustration is necessary for the production of exemplary illustrations in other fields of scientific education and publication, special students who wish to apply such techniques to a field of classical study other than medicine may be accepted in conformity with Paragraph B1 of this announcement. Only a limited number of applicants are selected each year for this training.

Applications for admission may be addressed to the Registrar, The University of Georgia School of Medicine, Augusta, Georgia.

ART AS APPLIED TO MEDICINE*

A. COURSES FOR MEDICAL STUDENTS

1. A course in the techniques of medical illustration: open to students in each year of medical school, to postgraduates and to members of the faculty, for one afternoon a week throughout the year.

2. Research workers who require illustrations for their research will be given guidance upon request.

B. COURSES FOR MEDICAL ART STUDENTS

1. Special courses to medical illustrators who desire to take advanced work in a particular branch, or to scientific illustrators who wish to apply the techniques of medical illustration to a field of classical study other than medicine. Minimum fee, \$40 per quarter.

2. A regular four-year course for beginners. This may be completed in four scholastic years, or in an accelerated course of thirty-six months. Applicants must have studied chemistry or biology, physics, zoology or

* (Extracted from the *Bulletin, The University of Georgia School of Medicine, Announcements, The Session 1948-1949*).

Significant toxic reactions occurred in 2 per cent of the patients treated. These consisted of 1 febrile reaction, 4 cases of leukopenia, and 1 case of agranulocytosis. The author now combines Lugol's solution with propylthiouracil from the beginning of treatment in all patients with toxic diffuse goiter. Although the exhibition of iodine somewhat delays the return of the B.M.R. to normal, it results in more prompt subjective improvement. No death occurred after thyroidectomy in the 300 patients treated with propylthiouracil.—*J.M.*

BOWERS, J. Z.: Hyperthyroidism occurring at an early age in dissimilar twins, *Ann. Int. Med.* 29: 935-941, 1948.

Case histories of a set of fraternal twins in whom hyperthyroidism developed at the age of 4 years 10 months and at 7 years 10 months are presented.—*J.M.*

CHAPMAN, E. M.: Treatment of Graves' disease with radioactive iodine, *West. J. Surg.* 56: 47-51, 1948.

The history of the application of radioactive iodine to the therapy of thyroid disease is reviewed, together with certain elementary physical characteristics of radioactive iodine having a half-life of 12 hours. From experience in treating 45 patients with toxic diffuse goiter, the author concludes that the 12-hour isotope is an effective single therapeutic agent.—*J.M.*

DANZIGER, LEWIS, and KINDWALL, JOSEF A.: Thyroid therapy in some mental disorders, *Dis. Nerv. System* 9: 231-241 (Aug.) 1948.

The authors review a considerable proportion of the relevant evidence and conclude that deficient oxidation in the brain is a potent factor in the causation of mental disorders. For the purpose of increasing the brain oxidation, thyroid substance or sodium-thyroxine was given to 5 psychoneurotic patients and to 19 psychotic patients (manic depressive and schizophrenic). Relatively large doses were employed. Recovery is reported in all the psychotic and in 2 of the psychoneurotic patients—results much better than those usually obtained in similar studies. Emphasis is laid on the desirability of prolonged treatment and of the probability that improved results might be obtained by the adjunctive use of other oxidation-promoters.—*R.G.H.*

DE ROBERTIS, E.: Proteolytic activity in the physiology, pathology and therapeutics of the thyroid gland, *West. J. Surg.* 56: 253-269, 1948.

By means of the freezing-drying technique, the author has demonstrated the presence of intracellular colloid which is the product of secretion and reabsorption inside the cells, and has shown that the release of colloid is always intracellular. In colloid extracted from single follicles in the rat thyroid, a proteolytic enzyme which digests a gelatin substrate was found. The enzyme activity increases after injection of TSH and after the administration of iodide for a short time. After treatment with iodide for a longer time, enzyme activity becomes subnormal. From these facts, the author postulates a theory of the enzymatic reabsorption of the colloid. He suggests that the process of release from thyroglobulin from the follicle involves an enzymatic mechanism which breaks down the large protein molecule and makes possible absorption by the cells. The rate of proteolytic activity of normal and pathologic human thyroid tissue was measured. In severe toxic goiter the proteolytic activity was found to be 96 per cent above that of the normal

Abstracts of CURRENT ENDOCRINE LITERATURE

Editor: ROY HERTZ. Collaborators: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS

PITUITARY

BLOOM, W.: Pituitary implications in hypertrophic pulmonary osteoarthropathy, *Ann. Int. Med.* 29: 361-370, 1948.

The case report of a patient with chronic hypertrophic pulmonary osteoarthropathy is presented. At autopsy a carcinoma of the lung with metastasis to the anterior lobe of the pituitary gland was found. The author suggests that overstimulation of the anterior pituitary by the metastatic lesion may have induced the hypertrophic osteoarthropathy. He analyzes other reports in the literature which suggest an endocrine basis for this syndrome.—J.M.

SCHWEITZER, FEDERICO L., and BAS, JOSÉ A.: Evaluation and differentiation of the various gonadotropic hormones by their action on the male toad, *Semana méd.* 55: 703-708, 1948.

Various gonadotropins were assayed comparatively on immature female rats and for their effect in producing release of spermatozoa in the toad *Bufo arenarum* Hensel following the technic described by Galli Mainini. A toad unit is defined as the minimum dose that produced a positive response in two-thirds of the animals, the total number of which varied widely but was never less than 7. One toad unit of chorionic gonadotropin corresponded to 38 international units. Crude extracts from pregnant mare serum or plasma and the purified hormone gave identical results showing 1 toad unit equivalent to 150 international units. It is concluded that either the ratio of FSH to LH in the pregnant mare hormone is not altered during purification or that the toad does not respond to FSH. This latter theory is in accord with the fact that the toad is not influenced by menopause urine. The difference in reaction may be used to differentiate chorionic and pregnant mare gonadotropin.—A.E.M.

THYROID

BARTELS, E. C.: Propylthiouracil; its use in the preoperative treatment of severe and complicated hyperthyroidism, *West. J. Surg.* 56: 226-235, 1948.

Propylthiouracil has been employed by the author in the preoperative treatment of 300 patients with moderate to severe hyperthyroidism. Daily doses of 200 mg. for toxic diffuse goiter and of 300 mg. for toxic nodular goiter were found to be uniformly effective.

ping in the radioactive iodine excretion between thyrotoxic and nonthyrotoxic individuals in the range 20 to 40 per cent. The finding of a low urinary excretion of radioactive iodine aided in establishing the diagnosis of Graves' disease in certain clinically equivocal cases. The finding of a high excretion assisted in excluding the diagnosis of Graves' disease in such conditions as alcoholism, anxiety, hypertensive cardiovascular disease, Parkinsonism, pheochromocytoma and thyrotoxicosis factitia.—*J.M.*

MEANS, J. H.: Present day trends in thyroid research, *West. J. Surg.* **56**: 65-71, 1948.

The trends in twentieth-century thyroid research are reviewed, indicating the fact that the types of research undertaken have become increasingly fundamental. Another tendency which is discernible is the application of an increasing number of disciplines to thyroid problems. Among the lines which the author regards as especially promising for future research are the phylogenetic development of the thyroid, the chemical nature of TSH, the mode of action of the thyroid hormone on its end organ, and the pathogenesis of the ophthalmopathy of Graves' disease.—*J.M.*

THOMPSON, W. O.; THOMPSON, P. K., and MANDERNACH, D. M.: Further observations on thiouracil and related substances in the treatment of toxic goiter, *West. J. Surg.* **56**: 270-277, 1948.

The authors believe that prolonged medical control of thyrotoxicosis with the thiouracils should be attempted in only 75 to 80 per cent of patients. This form of treatment is precluded in the remainder because of toxic reactions, size of the goiter and inability to control the disease adequately. However, from their data, it appears that the prolonged administration of the thiouracils can induce a remission in approximately 60 per cent of patients with thyrotoxicosis.—*J.M.*

WILLIAMS, R. H.: An evaluation of newer methods of treatment of thyrotoxicosis, *West. J. Surg.* **56**: 72-76, 1948.

The advantages and disadvantages of treatment of thyrotoxicosis with surgery, radioactive iodine and the thiouracils are reviewed. The treatment to be employed in a given case must be selected after careful evaluation of all the factors involved. Surgery is the best established form of treatment and the long-term results are the most predictable. It is the method of choice in cases in which the goiters are very large, particularly if they are nodular, and in cases in which there is a question of carcinoma. Factors to be considered against thyroidectomy are the discomfort and expense it entails, and the serious nature of the complications. Radioactive iodine therapy is extremely valuable in certain complicated cases of thyrotoxicosis, especially when toxic reactions or refractoriness to thiouracil develop. However, the harmful effects which it may ultimately produce cannot yet be estimated. The thiouracils produce a remission in more than 50 per cent of patients treated continuously for a year. If propylthiouracil be employed, toxic reactions are relatively infrequent. The author concludes that none of these forms of therapy is ideal.—*J.M.*



gland. In two cases of toxic adenoma, a difference of approximately 100 per cent was found between the proteolytic activity of the adenoma and that of the paranodular tissue. In colloid goiters the proteolytic activity was only 27.9 per cent of normal. It is suggested that the therapeutic action of iodine may depend, in part, upon its capacity to inhibit the proteolytic system. Thyroid inhibitors of the thiouracil type appear to act on special oxidative mechanisms of the cell, rather than through the proteolytic system.—*J.M.*

HEINEMANN, M.; JOHNSON, C. E., and MAN, E. B.: Serum precipitable iodine concentrations during pregnancy, *J. Clin. Investigation* 27: 91-97 (Jan.) 1948.

Serum precipitable iodine determinations were made in 43 pregnant women and were followed after delivery in 11. Of these 43 women, ranging in age from 21 to 44 years, 29 were normal, with no personal histories suggestive of metabolic disease. In this group, concentrations of serum precipitable iodine ranged between 6.2 and 11.2 gamma per cent, the majority being a high normal or above the upper limit of the normal range of non-pregnant women (8.0 gamma per cent). Elevated values were observed as early as three to six weeks after conception, did not increase during the subsequent course of pregnancy, and subsided soon after delivery. The 10 women who aborted or threatened to abort showed concentrations (2.8 to 5.8 gamma per cent) of serum precipitable iodine which were low for normal pregnancy. In cases of threatened abortion, the serum iodine concentrations increased when desiccated thyroid was administered, but remained in the lower range of values of normal pregnancy in some instances. In one hypothyroid patient, in whom an abortion seemed inevitable in the fourth month, values for serum precipitable iodine increased from 6.6 to 10.0 gamma per cent after administration of intravenous thyroxine for two days and an increased dosage of desiccated thyroid during the remainder of pregnancy resulted in an average value of 7.8 gamma per 100 cc; a normal infant was delivered at term. Higher concentrations of serum precipitable iodine than in normal pregnancy occurred in the four hyperthyroid women; in all of these, pregnancy was maintained and the hyperthyroidism controlled by Lugol's solution alone or in conjunction with thiourea.—*T.H.McG.*

HERTZ, J.: Studies on thyrotoxicosis, *West. J. Surg.* 56: 209-225, 1948.

Follow-up examinations were performed one to three years after thyroidectomy in 90 of 94 patients operated upon for thyrotoxicosis. Of 81 patients designated as immediately recovered, 93.5 per cent still were recorded as recovered; 2.6 per cent presented myxedema; 2.6 per cent, recurrent thyrotoxicosis; and 1.3 per cent, postoperative tetany. Of 7 patients discharged with hypothyroidism, 85.7 per cent had now recovered, whereas 14.3 per cent had myxedema. Of 5 patients discharged with persistent hyperthyroidism, 80 per cent had recovered and 20 per cent showed continuing hyperthyroidism. The significance of these findings is discussed.—*J.M.*

MCARTHUR, J. W.; RAWSON, R. W.; FLUHARTY, R. G., and MEANS, J. H.: The urinary excretion of radioactive iodine as an aid in the diagnosis of hyperthyroidism, *Ann. Int. Med.* 29: 229-237, 1948.

The mean urinary excretion of radioactive iodine by 30 nonthyrotoxic patients was 60 per cent and by 22 thyrotoxic patients, 25 per cent. There was considerable overlap-

Continued investigations into the androblastoma series which will be reported here have shown that there also occur morphologically well-defined forms of feminizing tumors, often very rich in lipoid, which because of their hormonal effect have hitherto been classified as belonging to the granulosa tumor group under the terms: folliculome lipidique (Lecène (5), Varangot (6), Plate (7)) or granulosa cell tumor of tubular or adenomatous type (Traut and Butterworth (8), Henderson (9), Dougal (10)), but which on the basis of the demonstrated congruence with androblastoma testis (Teilum 1946 (3)) can be classified with certainty as tubular androblastomas.

Several hitherto inexplicable facts concerning the hormonal effect of the testicular and ovarian androblastomas will be elucidated by these findings.

Androblastoma tubulare (lipoides) (estrogen-producing Sertoli cell tumor) of the human testis.

In my case of feminizing androblastoma testis in a man aged 53 years with gynecomastia (3) the tumor, measuring $6 \times 4 \times 3$ cm., was found mac-



FIG. 1. Feminizing androblastoma (tubulare lipoides) testis = tubular Sertoli cell tumor, showing morphological congruence with "folliculome lipidique" (Lecène). Hematoxylin and eosin stain. $\times 210$.

roscopically to be of an intensely yellow color on the cut surface. Posterosuperiorly a border, 4 to 5 mm. wide, of apparently normal testicular tissue was found. The *histological* examination showed a motley picture corresponding to the various stages of differentiation in virilizing arrhenoblastomas of the ovary, with all transitions from a diffuse blastema to differentiated massive cords and tubuli. In the greater part of the tumor the tubuli were highly differentiated with bright cylindrical cells, characterized by a very copious content of lipoid, so that the tumor tissue in

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ESTROGEN-PRODUCING SERTOLI CELL TUMORS (ANDROBLASTOMA TUBULARE LIPOIDES) OF THE HUMAN TESTIS AND OVARY. HOMOLOGOUS OVARIAN AND TESTICULAR TUMORS. III.*†

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IN PREVIOUS publications (1, 2, 3) two series of tumors (homologous) of the testis and the ovary have been established, showing complete morphological congruence in all forms of differentiation: (I) *The dysgerminoma series (gonocytoma)*, and (II) *the androblastoma series*, forming the basis of an exact *histogenetic* classification of several special forms of tumor of the ovary, the nature and histogenesis of which have hitherto been doubtful and based on a more or less subjective judgment. This applies, for example, to the so-called mesonephroma ovarii (Schiller 1939 (4)) which displays morphological congruence with embryonal testicular tumors originating from germ cells (2) belonging to the dysgerminoma series (gonocytoma).

The virilizing so-called "adrenal tumors" and "luteoma" must also be referred to the androblastoma series, particularly on the basis of transition forms demonstrated between these and the Leydig cells in arrhenoblastomas and on the basis of morphological congruence with morphologically different types of androgen-producing interstitial cell tumors in the testis (3).

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† This study was aided by a grant from the King Christian X Foundation.

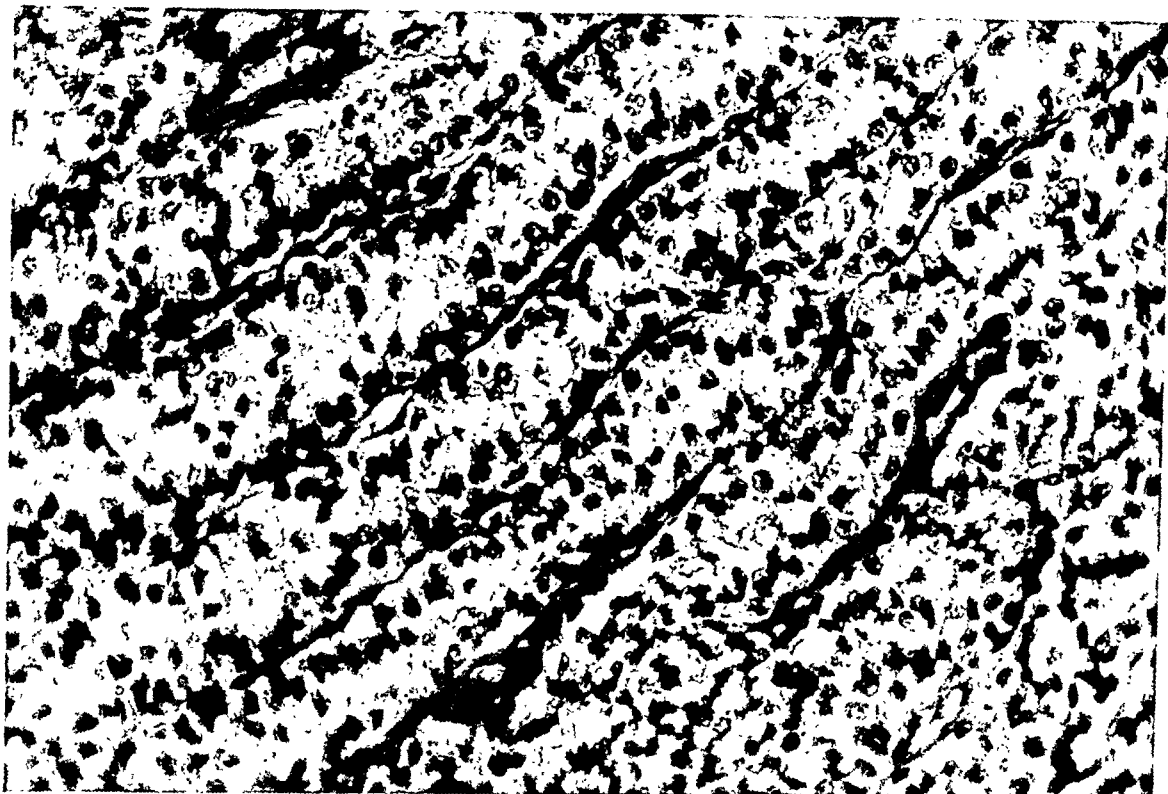


FIG. 3. Feminizing androblastoma testis showing the trabeculae cut parallel to their longitudinal axis. The linear bands of cells contain two rows of cylindrical cells with highly vacuolated cytoplasm and separated by connective tissue septa (*cf.* "folliculome lipidique" (Lecène)). v. Gieson-Hansen stain. $\times 370$.

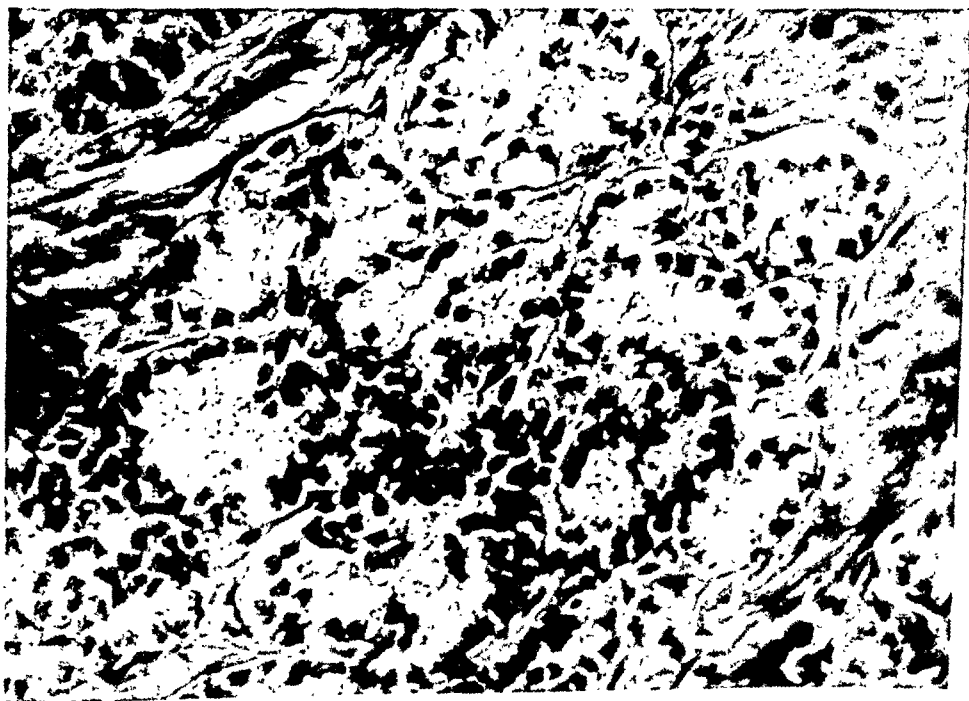


FIG. 4. The same testicular tumor. The trabeculae cut at right angles with their longitudinal axis. Radiate arrangement of the lipidic, highly vacuolated cells (*cf.* Hender-on's figures 2 D and 10 B, "hirsutism of adenomatous type"). v. Gieson-Hansen stain. $\times 310$.

these portions presented the histological picture of a tubular lipoid cell tumor. The tissue, in the parts where the trabeculae had been cut parallel to their longitudinal axis, was seen to consist of two rows of cylindrical cells with highly vacuolated cytoplasm (Figs. 1 and 3), whereas the trabeculae, when cut at right angles with their longitudinal axis, displayed a radial arrangement of the cells (Fig. 4) with transition to tubular cavities with cylindrical or cubical cells in a layer circumscribing a central cavity. A considerable lipoid content was demonstrated by means of osmium and sudan staining (Fig. 2).

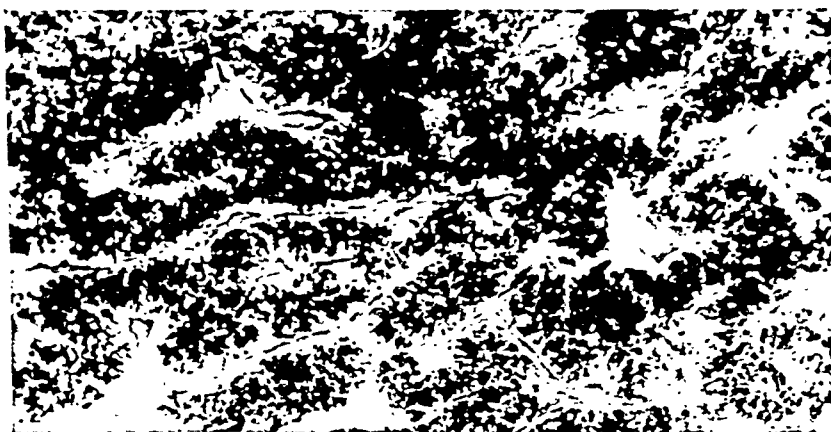


FIG. 2. Feminizing androblastoma of human testis (osmic acid stain). The tubuli consist of Sertoli cells with considerable lipid content (cf. Huggins & Moulder's figure 5 showing tubular Sertoli cell tumor of canine testis). $\times 240$.

Androblastoma tubulare (lipoides) (estrogen-producing Sertoli cell tumor) of the human ovary, hitherto considered as "folliculome lipidique" (Lecène) or as "granulosa cell tumor of tubular or adenomatous type" (Table 1).

a) *Three cases of "folliculome lipidique" (Lecène, Plate), collected by Varangot in 1937*

As previously described ((3), figs. 9 and 10 a and b), the lipidic tubular portions of the tumor of the testis were found in all respects to be identical with the so-called "folliculome lipidique" of the ovary of which Varangot in his monograph on granulosa cell tumors (1937, pages 140 to 151 (11)) gave a detailed description with eleven photomicrographs. This study was based on the 3 cases known till then, namely 2 cases observed by Lecène and the third by Plate. The first case, observed as early as 1910, was examined histologically by Lecène and presented by Christian before the

The cells seem to be distended by their cytoplasmic content and with sudan-staining they are seen to be filled with sudanophilic drops. The distribution of lipoid is not absolutely uniform and both in the ovarian tumor and in the corresponding testicular tumor, zones can be observed in which it is less copious. Lecène considered this accumulation of fat as being indicative of a luteinization. Plate agreed with Lecène and considered the hormonal function of the tumor tissue to be identical with that of the corpus luteum. Varangot, on the other hand, justly pointed out that accumulation of lipoid and luteinization are not synonymous. The cells have absorbed fat, but they are not of the same appearance as lutein cells. Functionally, no signs of lutein effect were found in these cases either. No signs of lutein influence were demonstrated either in Lecène's or in Plate's cases upon examination of the endometrium. Plate stated that the endometrium was hyperplastic. Varangot found that the hyperplasia suggested a folliculin-producing tumor. In the identification of the growth as a granulosa cell tumor he attributed greater importance to this functional test than to the morphological conditions, which, with regard to the occurrence of cylindrical cells, tubular and adenomatous structures, display such considerable and inexplicable deviations from the ordinary structure of the granulosa cell tumors.

b) *Two cases described by Traut and Butterworth (1937)*

In Traut and Butterworth's paper (8) photomicrographs are reproduced showing two ovarian tumors of the same type. In their figure 3 b, the structure is described as "tubular arrangement which is quite rare. Unquestionably granulosa, but simulating Pick's tubular adenoma," and their figure 4 b is labelled "general type of Lecène-folliculome lipidique."

Traut and Butterworth point out that there is no biological proof of progesterone effect of this so-called "folliculome lipidique," and they say as follows about the "tubular granulosa cell tumour": "Rarely the granulosa cells form a system of tubules in which single rows of cells arrange themselves about a central acinus as though it were a duct. Such an arrangement of cells might be thought to be related to some form of tubular adenoma, such as Pick's testicular tumor, were it not that one can usually prove beyond doubt that they are true granulosa cells."

c) *Henderson's case of "granulosa cell tumor of adenomatous type" (1942)*

Henderson's series (9) of 21 granulosa cell tumors and 9 theca cell tumors includes one case of "granulosa cell tumor of an adenomatous type" which is termed rare. The photomicrographs with cross-sections of bright tubuli with epithelial cells rich in lipoid (their figures 2 D and 10 B) are identical with our testicular androblastoma (see Fig. 4). Henderson's case was that

Société anatomique (12). Lecène observed another case in 1927, and Plate found the third in 1933.

The fact that, macroscopically, the tumor is of a yellow color indicates a high content of lipoid. Varangot described the ovarian tumor histologically

TABLE 1. TEN CASES OF ANDROBLASTOMA TUBULARE LIPOIDES OVARII OR TUBULAR SERTOLI CELL TUMOR

Author	Earlier diagnosis	Patient's age (yrs.)	Endocrine symptoms
1. Christian = Lecène I (1910)	Folliculome lipidique	21	Metrorrhagia
2. Lecène II (1927)	Folliculome lipidique	—	
3. Plate (1933)	Folliculome lipidique	23	Metrorrhagia, hyperplasia endometrii; later amenorrhea 7 months
4. Traut & Butterworth I (1937)	Tubular granulosa cell tumor	—	Not stated
5. Traut & Butterworth II (1937)	Folliculome lipidique	—	—
6. Henderson (1942)	Luteoma of adenomatous type	7	Metrorrhagia and pubertas praecox
7. Dougal (1945)	Granulosa cell tumor of tubular or adenomatous type	22	Metrorrhagia
8. Grevle (1936)	Granulosa cell tumor of special structure	26	Metrorrhagia
9. Pick (1905)	Adenoma tubulare	34	Metrorrhagia
10. Schickele (1907)	Adenoma tubulare	26	Metrorrhagia

as composed of "des bandes cellulaires tantôt rectilignes, tantôt flexueuses et festonnées, séparées par des travées conjonctives le plus souvent fines et lâches, parfois hyalinisées."

The characteristic pictures, dependent on whether the trabeculae are cut parallel or at right angles to their longitudinal axis, are identical with those described in the section on testicular androblastoma.

ation, being approximately the size of a chicken's egg and very easily enucleated, so that only a narrow border of ovarian tissue remained. On the cut surface the tumor was of a yellow color and of a rather soft consistency. Microscopically it was composed of cells arranged in gland-like or tubular formations. The epithelium was almost cylindrical. After operation the menstrual periods became regular. It appears from the photomicrographs reproduced that the tumor was of the same type as in the preceding cases, but less lipidic than "folliculome lipidique" Lecène.

Morphological congruence and common estrogenic effect of androblastoma lipidoides of the testis and the ovary (Table 2).

Eight cases of tubular or adenomatous, most frequently highly lipidic,

TABLE 2. RELATION BETWEEN DOMINANT CELL TYPE AND HORMONAL EFFECT IN IDENTICAL ANDROBLASTOMAS OF THE TESTIS AND THE OVARY

	Testis	Ovary
I. Estrogen-producing androblastoma:—Androblastoma tubulare lipidoides = Sertoli cell tumors		
Structure	chiefly tubular, lipidic	
Hormone production	estrogen	estrogen
Endocrine effect	feminizing; gynecomastia, impotence	feminizing; metrorrhagia, in children also pubertas praecox (Henderson)
Cases described	in man (Teilum, 1945-1946) in dog (Huggins & Moulder, 1945)	a number of tubular lipidic tumors, previously misinterpreted as folliculome lipidique (Lecène) or tubular granulosa cell tumor, and the pure tubular adenoma (see Table 1)
II. Androgen-producing androblastoma (besides Robert Meyer's virilizing arrhenoblastomas):—Androblastoma diffusum = Leydig cell tumors		
Structure	diffuse, often lipidic	
Hormone production	androgen	androgen
Endocrine effect	in adults: none in children: pubertas praecox	in adults: virilization in children: also pubertas praecox
Cases described	Masson's case and several others	tumors described as "adrenal tumors" and "luteomas"

of a 7-year-old girl with vaginal hemorrhage and pubertas praecox. The tumor was 4 cm. in diameter. The patient was alive and well six years after the operation. The endometrium had not been examined. The tumor was also termed "luteoma, adenomatous type" and was considered "completely luteinized," as the copious lipoid content was considered synonymous with luteinization in spite of the marked clinical signs of an estrogenic effect of the tumor.

d) *Dougal's case of "granulosa cell tumor of tubular or adenomatous type" (1945)*

A particularly instructive and thoroughly described case of this form of tumor of the ovary is the one reported by Dougal (10) in a 22-year-old woman with persistent uterine hemorrhage of seventeen months' standing. The diagnosis of granulosa cell tumor was established. At operation a solid, almost spherical, ovarian tumor, measuring 7.5 cm. in diameter, was removed. On the cut surface it was seen to be encapsulated, somewhat lobulated and of a *bright yellow color*. The histological picture was found to be "quite different" from the one usually found in granulosa cell tumors, as "the tumor was seen to consist of an encapsulated mass of perfectly formed tubules more or less divided up into lobules by incomplete septa, well supplied with blood-vessels. Lying over part of the circumference of the tumor and outside the capsule were the remains of the ovary proper containing Graafian follicles in various stages of development, a number of corpora albicantia but no recent corpora lutea. The general arrangement of tumor and ovary suggested that the tumor had not originated in the ovarian cortex, but in the medulla or hilum.

Microscopically the tubules were seen to be fully differentiated and to consist of a single layer of cylindrical cells with well marked inner boundaries and oval, deeply staining, peripherally placed nuclei. Sudan III stain revealed the presence of large amounts of lipid in the inner portions of the cells and in the lumina." Dougal considered the tumor to be an encapsulated, luteinized and hormonally active adenoma of the ovary. According to the clinical history the hormonal effect seems to have been *estrogenic*. The tumor was stated to be highly differentiated "but along unusual lines, the tubular arrangement being not unlike that present in Pick's testicular adenoma of the ovary which, however, is a virilizing tumor." The colored plates reproduced in Dougal's paper show complete conformity with similar sections in my case of feminizing androblastoma testis (Figs. 2 and 4).

e) *Greule's case 2 of granulosa cell tumor of peculiar character*

Greule's case (13) in a 26-year-old woman with metrorrhagia is of the same type. At the site of the right ovary a solid tumor was found at oper-

longitudinal axis the cells form long parallel bands with two rows of cells (Figs. 1 and 3), whereas in cross-sections a radial arrangement of the cells is seen (Fig. 4), often surrounding a distinct lumen.

Histogenesis and classification. The histogenetic classification of the tumor as a chiefly *tubular androblastoma* is based upon

1. *The morphological congruence* between "folliculome lipidique" of the ovary and the predominant sections of the feminizing androblastoma testis ((3), fig. 9 a and b), and

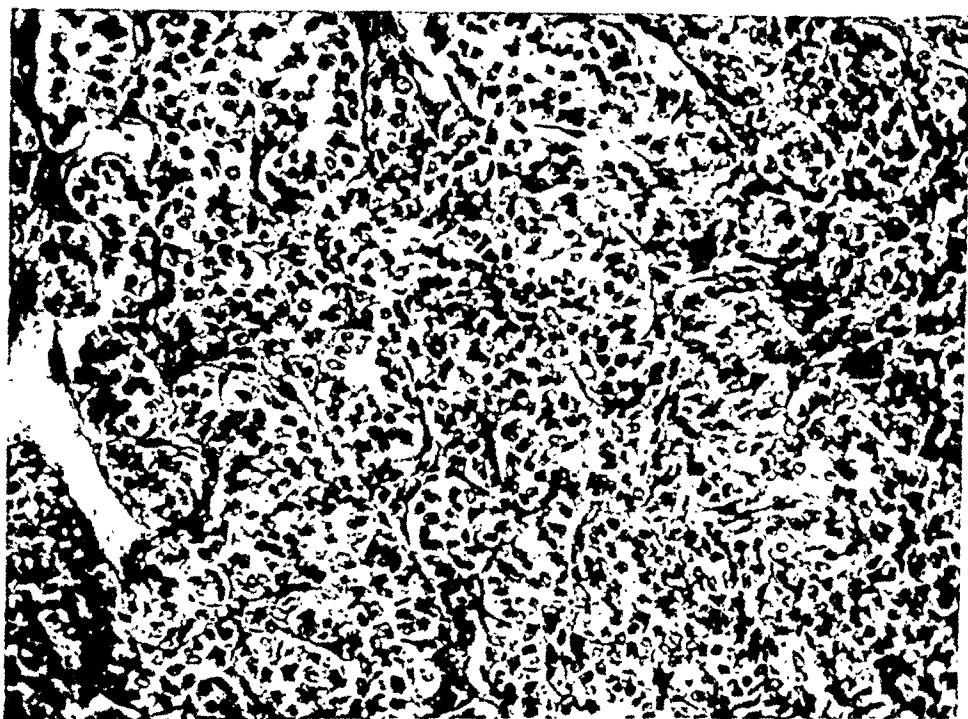


FIG. 5. Solid cords of undifferentiated Sertoli cells of androblastoma testis (cf. Figures 6 and 7). v. Gieson-Hansen stain. $\times 240$.

2. *the gradual transition* from solid cords and tubuli, which may be found in virilizing ovarian arrhenoblastomas (Fig. 6) as well as in the homologous testicular androblastomas (Fig. 5), to the highly differentiated lipidic tubuli with vacuolated cells (Figs. 1 and 3).

Thus there cannot be any doubt that the group of lipidic tubular ovarian tumors dealt with here and previously described as "folliculome lipidique" (Lecène's type) or granulosa cell tumor of tubular or adenomatous type—irrespective of their estrogenic effect—does not belong to the group of granulosa cell tumors, but represents ovarian tumors congruent with androblastoma tubulare (lipoides) of the testis (3) and must be classified as *androblastomas*, i.e., tumors which, like the arrhenoblastomas, originate histogenetically from male directed cell material.

ovarian tumors which, macroscopically, are yellow on the cut surface have thus been collected from the literature where they have hitherto been described as "folliculome lipidique" (5, 6, 7, 8, 11, 14) "completely luteinized" granulosa cell tumor (9) or granulosa cell tumor of tubular or adenomatous type (luteinized and hormonally active adenoma) (10).

On the basis of the demonstrated morphological congruence with the feminizing androblastoma testis (3) these tumors can with certainty be histogenetically classified as *androblastomas*, i.e., tumors which, like the virilizing arrhenoblastomas, originate from a testicular blastema (androblastema) in which a differentiation tending in the direction of Sertoli and (or) Leydig cells may take place, the hormonal effect of the tumor thus being dependent on predominance of either type of cell. The purely or chiefly tubular lipidic forms, like those dealt with here, will in principle correspond to the lipidic estrogen-producing tubular Sertoli cell tumors of the testis described in dogs (Huggins and Moulder (15)), as we may take it for granted that the lipidic cells in the tubular sections of the androblastomas represent Sertoli cells.

In the ovary, too, these tumors are hormone-producing. Although most of the cases have been looked upon as "folliculome lipidique" (Lecène) or as luteinized granulosa cell tumors of tubular type, no biological proof of progesterone effect of this form of tumor has been delivered (6, 8, 11). The clinical details such as metrorrhagia and, in Henderson's case in a 7-year-old girl, also pubertas praecox, and observations of endometrial hyperplasia in a few cases, indicate an active estrogen-production of the tumor (6). This form of tumor is highly differentiated and has proved to be benign in all the cases described. Several of the authors of the case reports emphasize the morphological resemblance to tubular adenoma of the ovary (7, 8, 10), but they all interpret the tumors as belonging to the granulosa cell tumors, as apparently they have attributed a greater importance to the function of the tumor than to the morphological conditions which, as pointed out by Varangot, display such considerable deviations from the ordinary structure of the granulosa cells.

It may be mentioned that during recent years the term "folliculome lipidique" has been used as a synonym for any luteinized granulosa cell tumor (Novak (16)), but that the papers reported here deal only with tubular tumors identical with Lecène's original type.

Macroscopically the tubular androblastomas are not very large. They are often of almost spherical shape, solid, of a yellow color on the cut surface and well encapsulated in many cases, with a border of ovarian tissue surrounding the tumor.

Histologically, more or less lipidic tubuli with bright vacuolated cylindrical cells are seen; where the trabeculae have been cut parallel to their

without impairment of the Sertoli or Leydig cells, whereas the secretion of F.S.H. is normal. This is taken as a sign that the Sertoli cells regulate the gonadotropic activity of the pituitary by means of a secretion product.

Testis tumors with estrogen effects have often been described in dogs (lit., see (15)). The dogs are sexually attractive to other males, the mammary glands are enlarged, with hypertrophy of the mammary papillae. Squamous metaplasia of the epithelium of the prostate and posterior urethra is usual. The site of estrogen production had not been identified before the work of Huggins and Moulder (15) in 1945. They say as follows about this question: The germinal epithelium of the gland is certainly not its source, as the tumors derived from the germinal epithelium (seminomas) do not cause squamous metaplasia of the prostate. That the Leydig cells should produce estrogenic hormone does not fit in with cases where interstitial cell neoplasms produce large amounts of the male sex hormones. An adult described by Masson (1943 (20)) with a malignant interstitial cell tumor had an extremely large titer of urinary androgen but no gynecomastia.

The hypothesis that these tumors of the canine testis are derived from Sertoli cells is based by Huggins and Moulder on histochemical study of the lipoids and estrogen extraction and assay. It appeared that all testis tumors of feminized dogs contained large amounts of lipoid—from 75 to 370 per cent more than normal canine testis. In 3 cases the neoplastic cells were arranged in tubular formation, and in 2 others abortive tubular formation was present. By definition, the cells of the testicular tubules with high lipid content are Sertoli cells. The estrogen content of these lipidic neoplasms was found to be considerable; in one of Huggins and Moulder's cases it was equivalent to 0.07 mg. per Kg. calculated as α -estradiol benzoate, "being twice the content of the ovaries at estrus and one-third of the amount in equine testis, the richest known source of estrogen." Estrogen could not be extracted from a Sertoli cell tumor that had not caused feminization.

When a morphological comparison is made between the Sertoli cell tumors in dogs and the feminizing and chiefly tubular androblastoma testis in man (Figs. 1 to 5) there can, in spite of all differences (*e.g.*, with regard to the degree of differentiation of the cells), be no doubt that the cord-shaped and tubular portions of the androblastomas are identical in principle with the structure of the Sertoli cell tumors in dogs, just as the cells of the cords and tubuli of arrhenoblastomas must be considered identical with the Sertoli cells of the testis. The considerable lipid content of the cells in the highly differentiated tubular portions also shows complete conformity (compare our Fig. 2 with Huggins and Moulder's fig. 5).

Cases of Sertoli cell tumors in dogs, with closely set solid cords without

Nature of the hormone-producing cells of androblastomas.

At first glance such an interpretation of these *estrogen*-producing ovarian tumors as tumors originating from a *testicular* blastema or *male* directed cell material may perhaps seem difficult to explain. In this connection, mention should be made of Huggins and Moulder's demonstration of estrogen production of highly lipidic, often tubular, Sertoli cell tumors of the testis in the dog (15).

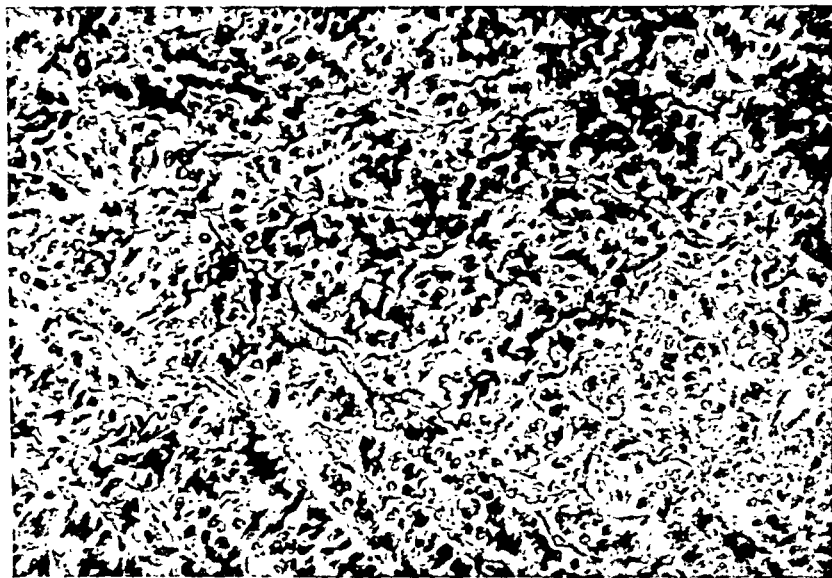


FIG. 6. Solid cords of undifferentiated Sertoli cells of virilizing arrhenoblastoma ovarii (cf. Figures 5 and 7). v. Gieson-Hansen stain. $\times 240$.

In 1918 Sand (17) advanced, with his vicario-theory, the view that under certain conditions the Sertoli cells take part in the hormone production of the testis.

In 1942 Klinefelter, Reifenstein and Albright (18) described a clinical syndrome (so-called Klinefelter syndrome) characterized by bilateral gynecomastia, aspermatogenesis without absence of Leydig cells, increased secretion of F.S.H. and hyalinization of the tubuli, so that both germinal cells and Sertoli cells were involved. These authors supposed that in addition to androgenic hormone from the Leydig cells the testis also produced another hormone similar to estrin from the tubuli, and they also pointed out that inhibition of formation of castration cells is not a physiological function of testosterone. In conformity therewith the syndrome described by Castillo, Trabucco and Balze (19) shows absence of germinal epithelium

or exclusively, tubular parts (containing Sertoli cells), the androgenic effect will fail to appear.

Ovarian tumors with dominance of tubular portions and a simultaneous marked accumulation of lipoid in their epithelium—similar to what is found in the most highly hormonally active Sertoli cell tumors in dogs—must then in advance be expected to exert an estrogenic effect.

This is just what we find in the cases referred to in the preceding pages—cases of tubular ovarian tumors with a high lipid content (metrorrhagia, pubertas praecox) which, however, has caused them to be misinterpreted

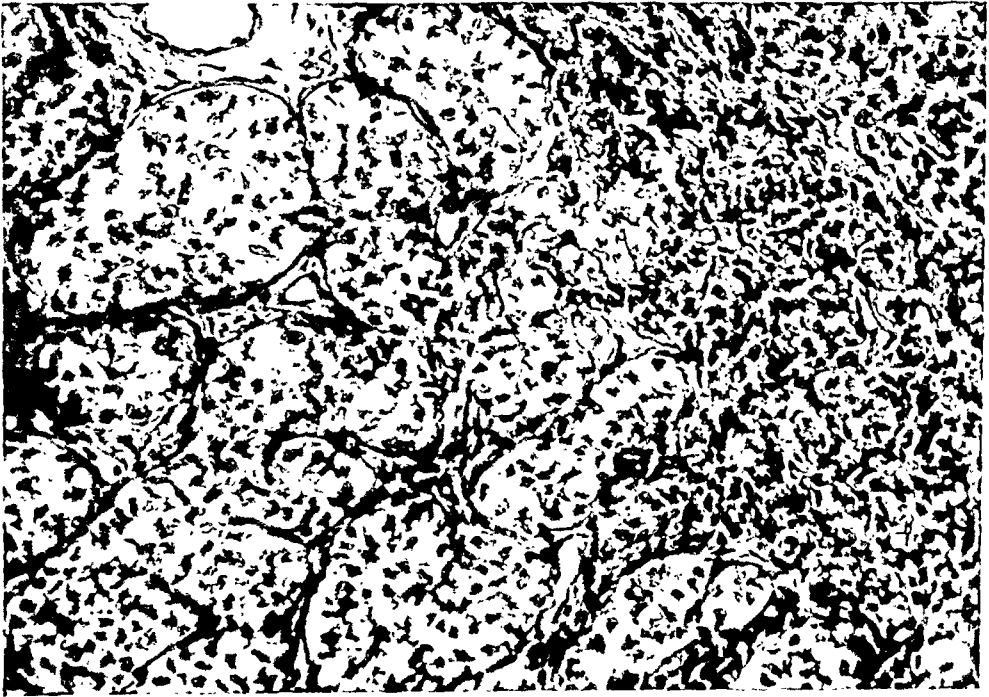


FIG. 8. Androblastoma testis. Differentiation of cords containing Sertoli cells in diffuse androblastoma. v. Gieson-Hansen stain. $\times 240$.

as granulosa cell tumors (folliculome lipidique (Lecène), granulosa cell tumor of tubular or adenomatous type), in spite of the fact that the morphological structure differs from the usual one characteristic of tumors of the granulosa cell group. The histological structure, on the other hand, is completely identical with that of androblastoma testis (Figs. 3 and 4).

In other words, owing to the occurrence of both androgen-producing Leydig cells and estrogen-producing Sertoli cells in the testicular androblastomas we can also expect to find both androgen- and estrogen-producing tumors in the series of homologous ovarian androblastomas.

This throws light on still another phenomenon which seemed previously inexplicable (Pedersen (21)), namely, that the androblastomas appear to be virilizing in women but feminizing in men.

distinct adenomatous structure (Fig. 7) are morphologically identical with corresponding parts of arrhenoblastoma ovarii (Fig. 6) and androblastoma testis (Fig. 5).

Thus tumors of the androblastoma series (including the arrhenoblastomas) may contain two different types of hormone-producing cells corresponding to Leydig cells and Sertoli cells (or their precursory stages) with production of androgenic and estrogenic hormones respectively (Fig. 8).

A testicular tumor with complete dominance of Leydig cells and production of androgenic hormone will give an increased output of this hor-

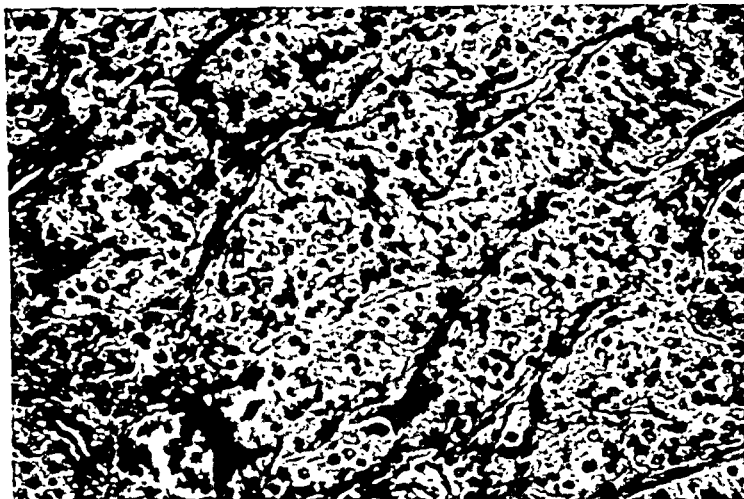


Fig. 7. Solid cords of Sertoli cells in Sertoli cell tumor of canine testis. (cf. tumors of human testis (Fig. 5) and of human ovary (Fig. 6)). Hematoxylin and eosin stain. $\times 240$.

none (as in Masson's case), but no clinical symptoms of endocrine disease. In the ovary, on the other hand, the homologous tumor will be clinically virilizing and correspond to tumors which have previously been incorrectly described as adrenal cell tumors or luteomas, but which are actually diffuse androblastomas homologous to Leydig cell tumors (Teilum (3)).

The virilizing effect of arrhenoblastomas of intermediary or diffuse type will also be dependent on occurrence and dominance of hormonally active Leydig cells or their precursory stages.

It has long been a well-known but inexplicable fact that the most highly differentiated arrhenoblastomas, by which has been understood the purely or chiefly tubular forms, most frequently have little or no hormonal (*i.e.* virilizing) effect. This is, however, quite simply explained by the occurrence of those types of cell in which the production of androgenic or estrogenic hormone respectively is possible. If the tumor tissue contains chiefly,

different hormonal production. The feminizing tubular lipid-cell tumor and the virilizing Leydig cell tumor (mistaken in the ovary for "adrenal tumor," "luteoma") both represent highly differentiated, hormone-producing androblastomas with dominance of Sertoli cells or Leydig cells respectively. In the undifferentiated diffuse forms (so-called sarcomatous type) it is, as a rule, impossible to decide whether the cell type represents mother cells of Sertoli or of Leydig cells. In a case such as the one reported by Østergaard (26)—chiefly diffuse feminizing testicular tumor, described as "presumably aberrant adreno-cortical tumour"—a radiating arrangement in parts forming small alveoli could, however, in addition to the cytological findings, indicate that this was a case of undifferentiated Sertoli cell tumor.

Terminology.

The term *androblastoma* (androma) was introduced by me to designate a series of tumors in the testis, homologous (and morphologically congruent) with the arrhenoblastoma series defined by Robert Meyer (27). As these tumors of the testis were not virilizing, the term arrhenoblastoma could obviously not be applied to them. Continued examinations of homologous tumors (3) showed, however, that Robert Meyer's group had to be extended so as to comprise also the diffuse so-called "adrenal cell tumors" (=Leydig cell tumors) with virilizing effect and the so-called "folliculome lipidique" (Lecène, Plate) with estrogenic effect, as they both displayed morphological congruence with the testicular androblastomas. Thus the histogenetically defined series of tumors also comprises types of tumor in the ovary which, in spite of their origin from male directed material, are not only without any virilizing effect but, in the case of the purely tubular forms, are estrogenic. It seems to me to be consistent to term the whole of this group of tumors androblastomas—of the ovary as well as of the testis—and to leave Robert Meyer's term arrhenoblastoma for the group which is, in addition, characterized clinically as virilizing.

As far as the highly differentiated pure, or chiefly pure, forms mentioned in the preceding pages are concerned, the terms tubular Sertoli cell tumor (15) or ovarian Leydig cell tumor may be employed, but to illustrate the histogenesis and the continuity between the individual forms of differentiation in the testis and the ovary the term androblastoma seems to be adequate.

SUMMARY

1. In continuation of previous studies on identical tumors of the ovary and the testis a morphological congruence is demonstrated between the feminizing androblastoma (tubulare lipidoides) testis and feminizing

This is simply due to the fact that only androblastomas with preponderance of estrogen-producing Sertoli cells give rise to clinical symptoms of endocrine disease in males, whereas the corresponding tumor of the ovary may be either virilizing (in case of dominance of Leydig cells) or feminizing—just like the testicular tumor (in case of dominance of Sertoli cells). As demonstrated above, it has, however, in the latter case been misinterpreted as “folliculome lipidique” or tubular granulosa cell tumor. As will be seen, only the heterologous hormonal effect has been recognized clinically.

According to these findings it was to be expected that pure, so-called “tubular adenomas” of the ovary are not only clinically without any virilizing effect, which, as already mentioned, is well-known, but would be found in a number of cases to be estrogen-producing, like granulosa cell tumors; and, again like these, associated with metrorrhagia in some cases. An examination of the literature on the subject proves that this is so. Both the case described by Pick (1905 (22)) and the one reported by Schickele (1907 (23)), both cases of tubular adenoma, had marked uterine hemorrhage in the anamnesis, a hitherto completely unnoticed and also inexplicable symptom in tubular adenoma.

In this connection mention may also be made of a case (reported by Goldberg and Maxwell (1947 (24))) of bilateral arrhenoblastoma without masculinization in a 19-year-old girl presenting a paradoxical picture of primary amenorrhea, tall eunuchoid build, large breasts, poorly developed external genitalia with rudimentary vagina, congenital absence of uterus and cervix, absent axillary and scant pubic hair and a high gonadotropin titer. Operation revealed bilateral, poorly developed gonadal structures composed of tissue grossly resembling adrenal cortex, proved to be a highly differentiated type of arrhenoblastoma. Careful search revealed no evidence of ovarian tissue. It is added that “the source of the estrogens responsible for the breast development remains a mystery.”

The ovarian tumors are identical with the estrogen-producing androblastoma tubulare lipidoides and in all probability responsible for the breast development.

The high lipid content in the tubular hormone-producing androblastomas in man and dog is in good conformity with Greenblatt, Greenhill and Brown's (1939 (25)) demonstration of the fact that incretory tumors differ from the nonincretory tumors in that the former have a consistently greater phospholipid and cholesterol content than the latter.

According to what has been set forth in the preceding pages, it is incorrect to consider the tubular androblastoma as the only highly differentiated form. The nontubular portions of the androblastomas may contain equally differentiated cells, namely Leydig cells, but with a qualitatively

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ovarian tumors, which have previously been described as "folliculome lipidique" or granulosa cell tumors of tubular or adenomatous type.

2. These ovarian tumors can thus be classified as androblastomas, *i.e.*, tumors originating from a testicular blastema in which a differentiation tending in the direction of Sertoli and (or) Leydig cells may take place.

3. The estrogenic effect of androblastoma tubulare lipidoides of the human ovary as well as of the testis is explained by dominance of tubular portions (Sertoli cells) in accordance with Huggins and Moulder's demonstration of estrogen-producing, lipidic (often tubular) Sertoli cell tumors in dogs. Similarly, the hormonal effect of virilizing androblastomas, *i.e.*, arrhenoblastomas and Leydig cell tumors (so-called "adrenal cell tumors" and "luteomas"), is supposed to depend on a dominance of androgen-producing Leydig cells.

4. Through the histogenetical classification of the androblastomas the following, hitherto inexplicable, points are elucidated:

a) That the "most highly differentiated" (*i.e.*, chiefly tubular) arrhenoblastomas have the least pronounced (frequently lacking) hormonal (*i.e.* virilizing) effect.

b) The apparently different hormonal effect of the androblastomas in either sex (feminizing in males, virilizing in females).

c) The hitherto unnoticed condition that the purely tubular testicular adenoma of the ovary is often associated with metrorrhagia.

d) The estrogen effect (development of the breasts) in cases of bilateral, yellow, purely tubular "arrhenoblastomas" with simultaneous aplasia of the genital system, including the ovaries.

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2) A decrease of hypertension or albuminuria after delivery, if these signs had persisted despite therapy up to delivery.

Women were considered as having "minimal or no edema" when they either had no clinical edema at all or had only minimal pitting edema of their feet and ankles. Women were considered as having "excessive edema," when their face, hands, and feet were swollen considerably and when they showed large postpartum losses in weight.

METHODS

All studies on pregnant women were made between the thirty-first and fortieth weeks of pregnancy.

Twelve-hour overnight urine samples were collected in bottles containing 5 cc. of toluene and extracted within eight hours of the end of the collection. Aliquots of 150 cc. of the urine were acidified with 12 N H_2SO_4 to pH 1 as determined with a glass electrode. The aliquots were then immediately extracted once with 300 cc. of redistilled ethyl acetate and once with 150 cc. of the ethyl acetate. The ethyl acetate extracts were combined and extracted twice with 90 cc. portions of 0.1 N H_2SO_4 , then twice with 150 cc. quantities of 2 N NaOH and then 5 times with 75 cc. portions of distilled water.

The purified ethyl acetate extracts were then evaporated to dryness *in vacuo*, the surrounding water bath never exceeding a temperature of 42° C.

The residue was brought into solution with 3 cc. of absolute ethyl alcohol, and corticosteroids were determined, utilizing in the main the procedure of Loewenstein (19). This involves cleavage of the 20, 21-ketol side chain of the corticosteroid by oxidation with periodic acid, with the release of formaldehyde (20). Formaldehyde is then determined by the colorimetric method of MacFadyen (21).

To the residue dissolved in 3 cc. of absolute ethanol, 20 cc. of water was added, and quickly thereafter 4 cc. of the periodate solution (0.01 M KIO_4 dissolved in .036M H_2SO_4 solution). The oxidation mixture was immediately shaken and allowed to react for 20 minutes at room temperature with occasional shaking.

Then 0.5 cc. of freshly prepared 6 per cent SnCl_2 solution dissolved in 1:1 concentrated HCl was added to terminate oxidation. A white precipitate appeared on addition of the Sn Cl_2 solution.

The mixture was then filtered through Whatman #12 paper to prevent turbidity in some specimens and a 15 cc. aliquot of the filtrate was placed in a standard 25 × 200 mm. "NPN" tube. The tube was immersed in a bath of ice water and 20 cc. of concentrated H_2SO_4 was slowly added to the tube. Then 1 cc. of a 7 per cent chromotropic acid solution was added and the contents mixed with a footed stirring rod.

The tubes were then placed in a boiling water bath for 30 minutes, were brought to room temperature and the solutions were read in an Evelyn colorimeter, using an Evelyn #565 filter.

An aliquot of 150 cc. of distilled water was always carried through the entire analytic procedure and this solution served as a blank.

It is realized that this procedure is not necessarily specific for "corticosteroids," such as the crystalline, physiologically active steroids that have been isolated from adrenal glands. Any compound with a free, terminal, vicinal glycol or hydroxyketone group will also release formaldehyde on HIO_4 oxidation. Furthermore, it is possible that there are compounds present other than formaldehyde which produce a lavender color when treated with chromotropic acid. They are said by MacFadyen to be few (21).

CORTICAL STEROID EXCRETION IN EDEMA OF PREGNANCY, PRE-ECLAMPSIA, AND ESSENTIAL HYPERTENSION*

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THE syndrome seen in desoxycorticosterone intoxication is in many respects strikingly similar to toxemia of late pregnancy (pre-eclampsia and eclampsia). In both syndromes one may find hypertension (1, 2, 3, 4), edema (2, 3, 4, 5), and albuminuria (2, 4, 6).

In both syndromes the hypertension is greatly alleviated by drastic dietary sodium restriction (7, 8, 9). In both syndromes the hypertension and albuminuria may be markedly increased by an excessive sodium intake in the diet (6, 7).

Excessive DCA administration to animals usually causes atrophy of the adrenal cortex, presumably because of an inhibition of corticotropic hormone secretion by the anterior pituitary (10, 11, 12). Paralleling the DCA findings, Fauvet found abnormally small adrenals in women dying of eclampsia (13) and reported a decreased concentration of corticotropic hormone in the serum of eclamptic women (14).

Women with essential hypertension have a greatly increased susceptibility to toxemia of pregnancy (15a). They are also more susceptible to the pressor effects of DCA (16).

Renal disease which produces hypertension also increases susceptibility to toxemia. Unilateral nephrectomy (17) and Masugi nephritis (18) in animals increase the toxic effects of DCA.

The foregoing considerations suggested the possibility that toxemia of pregnancy may be related to an increased concentration of desoxycorticosterone-like steroids in the body fluids or cells. Therefore, urinary corticosteroid excretion was measured in normal and toxemic pregnant women. Determinations were also made on normal individuals and on patients with essential hypertension.

Criteria for clinical classification

The following criteria were satisfied before the diagnosis of pre-eclampsia was made:

1) The occurrence in late pregnancy of a definite increase in diastolic blood pressure or proteinuria over the pre-pregnant level.

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to be a sound addition to this method and it is planned to adopt this feature in future determinations. However, even without the distillation it appears that our procedure analyzes essentially the same compounds as Daughaday and co-workers (23), since our normal values are almost identical with theirs.

With periodic acid treatment, each mole of corticosterone or desoxycorticosterone yields 1 mole of formaldehyde. We have arbitrarily chosen to express our results as milligrams of desoxycorticosterone excreted per 12 hours. This figure is arrived at by multiplying the milligrams of formaldehyde found, by a factor 11.0, since a mole of desoxycorticosterone weighs 11.0 times as much as a mole of formaldehyde.

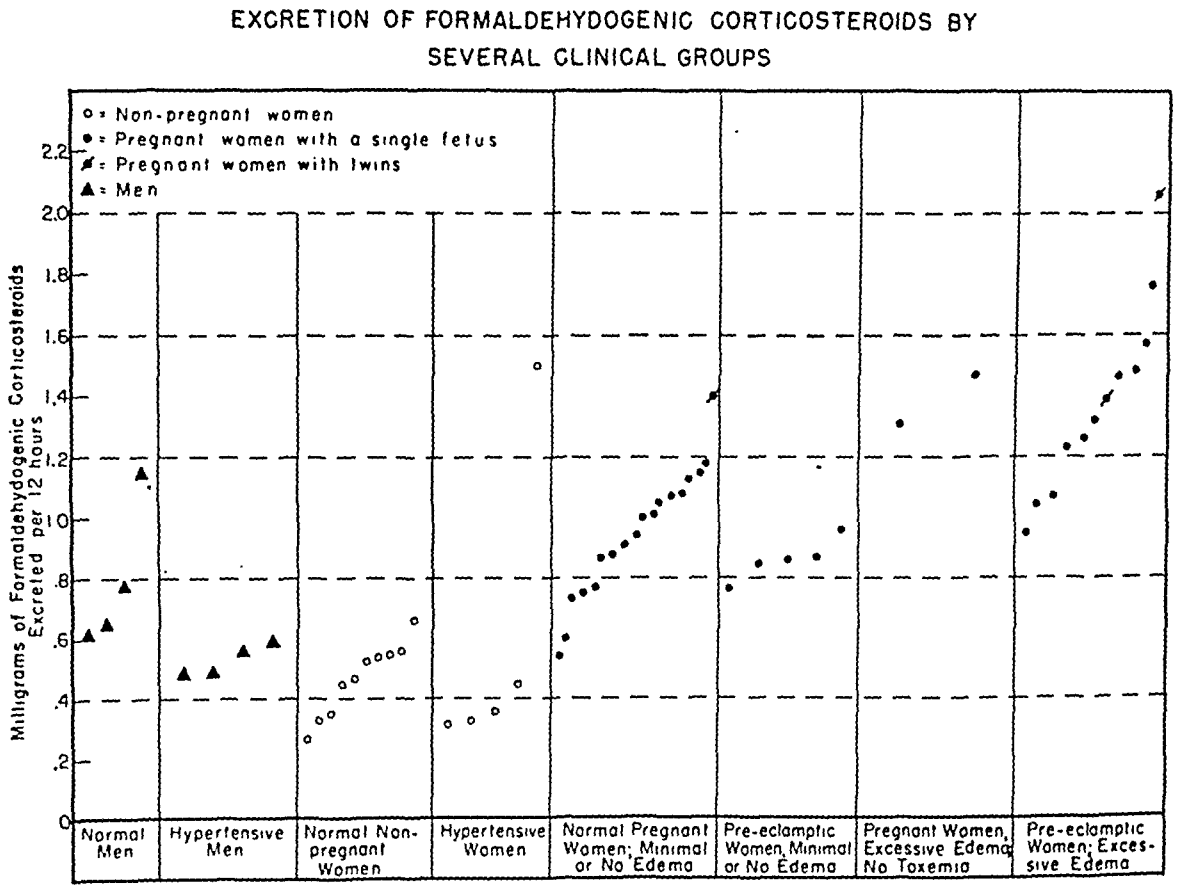


FIG. 1

Following the suggestion of Daughaday *et al.* (24), the cortical steroids will be called “formaldehydogenic corticosteroids” throughout the rest of the paper. To save space this will usually be abbreviated to “HCHO” corticosteroids.

RESULTS

According to the data presented in Table 1 and Figure 1, normal pregnant women in the last trimester excreted about twice as much “HCHO” corticosteroid per 12 hours as normal non-pregnant women.

The results also show that 2 of the 3 women with twins excreted con-

Because of this possible nonspecificity, it was thought that any simple procedures which would remove contaminating substances from the final residue without greatly diminishing the neutral corticosteroids would be desirable additions. For this reason the acid and alkali washes of the ethyl acetate extracts were included in the procedure.

Ethyl acetate was chosen for a solvent because it will more readily extract polar, highly hydroxylated corticosteroids such as 17-hydroxycorticosterone or steroids with solubility properties like the sodium-retaining steroid recently isolated by Thatcher and Hartman (22). Moreover, its low density compared to water facilitates washing (in contrast to chloroform).

By extracting with relatively large volumes of ethyl acetate, emulsions were avoided, even in urines containing considerable protein.

The color interference mentioned by Daughaday *et al.* (23) was not encountered, and it is possible that the interfering substances were removed in the alkali washes, which were seen to extract a brown pigment from the ethyl acetate fraction.

The formaldehyde distillation procedure described by Daughaday *et al.* (23) appears

TABLE 1. EXCRETION OF FORMALDEHYDOGENIC CORTICOSTEROIDS (MG./12 HRS.)
BY PREGNANT AND NON-PREGNANT FEMALES

Normal women	Normal pregnant women with minimal or no edema	Pre-eclamptic women with minimal or no edema	Pregnant women with excessive edema but without hypertension or albuminuria	Pre-eclamptic women with excessive edema
.27	.54	.77	1.31	.95
.33	.60	.85	1.47	1.04
.35	.73	.86		1.07
.45	.75	.87		1.23
.47	.77	.96		1.26
.53	.87			1.32
.54	.88			1.39 (twins)
.55	.91			1.46
.56	.94			1.48
.66	1.0			1.57
	1.01			1.76
	1.05			2.06 (twins)
	1.07			
	1.08			
	1.13			
	1.15			
	1.18			
	1.40 (twins)			
Mean				
.47	.95	.86	1.39	1.38

TABLE 4

Difference between means	Standard error of difference between means	T value	Odds against the observed differences between means being due to chance
Group II-Group I .908 - .471 = .437	.084	5.2	2,000,000 to 1
Group III-Group II 1.327 - .908 = .419	.145	2.89	250 to 1

value. In Table 2, all the toxemic women with a single fetus and a roughly equivalent amount of excessive edema are divided into three clinical groups in ascending order of clinical severity as judged by the degree of hypertension. The values in the table show that increasing levels of blood pressure in toxemia are not associated with increased "HCHO" corticosteroid excretion.

On the whole the data indicate that late pregnancy itself is associated with an increased "HCHO" corticosteroid excretion, which is even further increased in women having excessive edema in late pregnancy.

Table 3 shows the mean "HCHO" corticosteroid excretion, with its standard deviation, for each of these three groups: 1) normal non-pregnant women, 2) all pregnant women with minimal or no edema, and 3) all preg-

TABLE 5. EXCRETION OF FORMALDEHYDOGENIC CORTICOSTEROIDS (MG./12 HRS.)
BY NORMAL AND HYPERTENSIVE SUBJECTS

Normal women	Hypertensive women	Normal men	Hypertensive men
.27	.32	.62	.49
.33	.33	.65	.49
.35	.36	.78	.56
.45	.45	1.15	.59
.47	1.50		
.53			
.54			
.55			
.56			
.66			
Mean .47	.59	.80	.53

TABLE 2. FORMALDEHYDOGENIC CORTICOSTEROID EXCRETION (MG./12 HRS.) OF PREGNANT WOMEN WITH EQUIVALENT AMOUNTS OF EXCESSIVE EDEMA AND VARYING DEGREES OF PRE-ECLAMPSIA (WOMEN WITH TWINS NOT INCLUDED)

Mild pre-eclampsies		Moderate pre-eclampsies	Severe pre-eclampsies
1.07		1.04	.95
1.26		1.23	1.76
1.32			
1.46			
1.48			
1.57			
Mean	1.36	1.14	1.36

siderably more "corticosteroid" than any single-fetus woman in her clinical group.

The mean corticosteroid excretion of women with toxemia and excessive edema was about 45 per cent greater than that of the normal pregnant women with minimal or no edema.

However, the corticosteroid excretion of toxemic women without excessive edema was no greater than that of normal pregnant women without edema.

Two cases of so-called "excessive edema of pregnancy" without hypertension or albuminuria showed an increased excretion value similar to that seen in women with excessive edema and toxemia.

In cases with equivalent amounts of edema we found no correlation between the blood pressure level and the "HCHO" corticosteroid excretion

TABLE 3

Group No.	Type of subject	No. in group	Average formaldehydrogenic corticosteroid excretion (mg./12 hrs.)	Standard deviation of groups
I	Normal non-pregnant women	10	0.471	.199
II	Pregnant women with minimal or no edema	22	0.908	.262
III	Pregnant women with excessive edema	12	1.327	.464

*Relationship between "HCHO" corticosteroid excretion
and the adrenal cortex*

The "HCHO" corticosteroids measured were *excreted* and may very well not have been *secreted* in the body as such. They may be only the metabolites of steroid hormones which are actually secreted.

However, others have shown that in the non-pregnant individual the "HCHO" corticosteroid excretion level is a valid index of the secretion rate of adrenal cortical hormones, such level being elevated in Cushing's syndrome and other hyperadrenal states and being decreased in Addison's disease (24). In support of this we found a very high excretion of "HCHO" corticosteroids (1.7 mg./12 hrs.) in a man with a severe burn, a condition which is usually accompanied by adrenal cortex hypersecretion (25).

The maternal adrenal cortex is hypertrophied and richly laden with lipoids in late pregnancy. Venning has reported sizable increases in the secretion of "glycogenic corticoids" (26) and phosphomolybdate-reducing lipoids (27) in that stage of pregnancy. These findings indicate that the maternal adrenal cortex is very likely in a state of hypersecretion in late pregnancy and is probably the source of a large fraction of the increased "HCHO" corticosteroids we found to be excreted at that time.

There is no direct evidence that any endocrine gland other than the maternal adrenal cortex contributes to the "HCHO" corticosteroids formed in the urine. However, it has been reported that women with Addison's disease experience a marked relief of symptoms in the last months of pregnancy (28, 29). Their need for substitution therapy diminishes or disappears altogether, only to return at the termination of pregnancy (30). These findings suggest that in late pregnancy, sources other than the maternal adrenal cortex normally show an increased secretion of substances whose effect is similar to that of the adrenal cortex hormone. It is quite possible that these corticomimetic hormones may partially contribute to the increased "HCHO" corticosteroids excreted in late pregnancy. Studies of the "HCHO" corticosteroid excretion in pregnant women with Addison's disease would furnish valuable information concerning this possibility.

*Relationship of "HCHO" corticosteroid excretion
and pre-eclamptic hypertension*

The "HCHO" corticosteroid excretion could not be correlated with increases in blood pressure seen in pre-eclampsia. However, the findings do not exclude the possibility that a steroid not measured by this method may be fundamentally important in the causation of the hypertension and albuminuria of pre-eclampsia and eclampsia.

It is not known what effect the decreased glomerular filtration often

nant women with excessive edema. Since twin pregnancy probably increases the "HCHO" corticosteroid excretion and constitutes a separate variable, all women with twins were excluded from these groups. The mean "HCHO" corticosteroid excretion of Group III was 46 per cent greater than that of Group II.

Table 4 shows the difference between the means of Groups I and II and of Groups II and III, along with the standard error of these differences, and the odds against the observed differences being due to chance. We believe that the observed differences between these means are significant.

Figure 1 and Table 5 show the "HCHO" corticosteroid excretion value in hypertensive men and women as well as in normal men and women. Except for one hypertensive female, the patients with essential hypertension had no greater "HCHO" corticosteroid excretion than normal subjects.

DISCUSSION

Relationship between edema of pregnancy and the extra "HCHO" corticosteroid excretion associated with edema of pregnancy

Dexter and Weiss made a very careful and excellent clinical study of the edema associated with pregnancy. They concluded that "the etiology of the generalized edema of pregnancy cannot be explained by the well recognized mechanical causes of edema formation such as increased hydrostatic pressure in the capillaries, increased capillary permeability to protein, or hypoproteinemia. The edema is not due primarily to cardiac failure, anemia, vitamin B₁ deficiency, myxedema, or excessive ingestion of sodium salts, although many of these factors may at times play an important secondary role. By exclusion, a primary humoral etiology is suspected" (15b).

Earlier in this paper it was pointed out that DCA and other adrenal products can cause sodium retention and edema (1, 2, 3, 4, 5, 8, 16). We have also shown in the preceding section that pregnant women with edema have an increased excretion of "HCHO" corticosteroids. These interrelated facts strongly suggest that the extra corticosteroids or their precursors are the actual cause of the sodium retention and edema.

This concept cannot be considered as proved until all the actual hormones secreted during pregnancy and their metabolites have been identified and assayed. There may be other substances with edema-forming properties that are present in extra large amounts in edema of pregnancy. Certainly increased excretion rates of estrogens and pregnanediol are not associated with edema of pregnancy.

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seen in pre-eclampsia has on the renal clearance of the "HCHO" corticosteroids.

Relationship of "HCHO" corticosteroid excretion and essential hypertension

"HCHO" corticosteroid excretion was also not abnormal in most patients with essential hypertension. This is fairly good evidence against marked adrenal cortex hypersecretion in most patients with essential hypertension. However, it does not exclude the possibilities that 1) small increases in adrenal cortex secretion or 2) normal rates of secretion with alteration in the metabolism of the hormone after it is secreted, may be importantly involved in the pathogenesis of essential hypertension.

SUMMARY

1. Corticosteroid excretion in urine was estimated by a modified Loewenstein method.

2. Pregnant women in the last trimester excreted twice as much corticosteroid as non-pregnant women.

3. Pregnant women with excessive edema excreted about 46 per cent more corticosteroid than pregnant women with minimal or no edema. This difference is statistically significant.

4. The extra corticosteroids associated with edema of pregnancy or their precursors may perhaps be the cause of the renal retention of sodium and water producing the edema.

5. Women with twins showed a greater corticosteroid excretion than women with a single fetus.

6. The level of corticosteroid excretion could not be correlated with the elevation of blood pressure seen in pre-eclampsia.

7. Most patients with essential hypertension showed a normal corticosteroid excretion.

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THERAPEUTIC STUDIES IN HYPERTHYROIDISM: PROPYLTHIOURACIL

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INTRODUCTION

THE new methods of treating hyperthyroidism with the antithyroid drugs and radioactive iodine represent no fundamental change from the ancient methods of attack upon the disease. They are, respectively, merely forms of chemical and radiation thyroidectomy and, therefore, are no more functional than the method employed by our grandfathers of destroying the thyroid by the injection of boiling water.

The ideal treatment for hyperthyroidism would be based on a knowledge of the disorder causing the disease and a procedure to correct that disorder, returning the thyroid to its normal position in metabolic physiology rather than removing it from the endocrine chain. The possibility of such a solution is clinically demonstrable (1). For example, in the case of Mrs. L., shown in Figure 1, a puerperal hyperthyroidism of severe and unmistakable degree disappeared during the endocrine reaction induced by weaning her baby. A similar restoration of normal relations is shown in Figure 2, in which Miss Grace DeF. with moderately severe hyperthyroidism returned to normal after a few injections of chorionic gonadotropin.

PATHOLOGY

The antithyroid drugs derived from thiouracil do not seem to promote a restoration to normal but rather exaggerate the pathology of the disease, while paradoxically frustrating the formation of an increased amount of thyroid hormone. The intense hyperplasia resulting from the prolonged use of propylthiouracil is well illustrated in Figure 3, which shows a specimen taken from the gland of a patient who had received the drug for eight months (Miss L. S.). The small accumulation of colloid is due to the preoperative use of iodine: otherwise, as has been well shown by others, no colloid would be seen. The pathology resulting in this case may well be

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related to the fact that the disease in this patient was not controlled. The elevated blood iodine and the symptoms of thyrotoxicosis persisted even during the prolonged use of the drug (Fig. 9). It is conceivable that in patients cured by propylthiouracil a return to normal thyroid histology would be seen, but since in such cases no thyroidectomy is performed the opportunity to study the gland does not occur. The possibility that this involuted condition of the gland might be generally found is indicated in one case reported by Frisk (2).

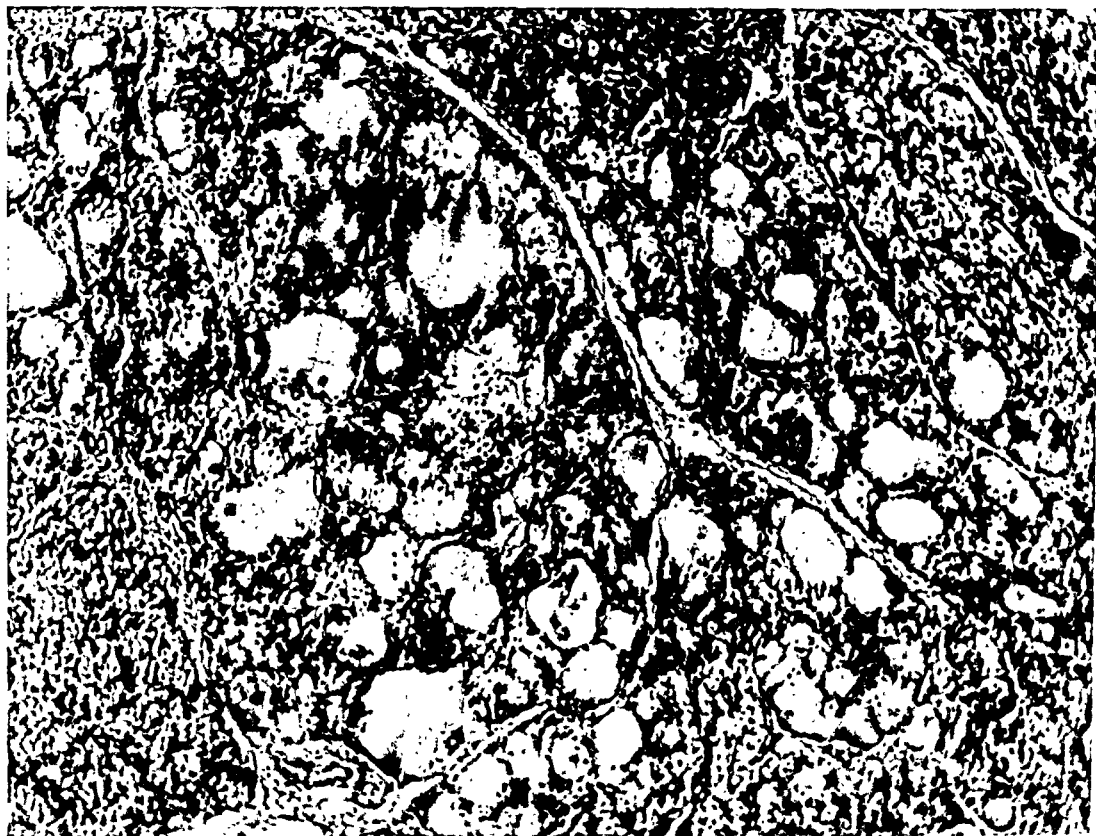


FIG. 3. Thyroid tissue removed at operation from Miss L. S. after eight months' administration of large doses of propylthiouracil. Iodine was administered for several weeks preoperatively. Intense hyperplasia and desquamation are shown (case 11, Fig. 9).

PROCEDURE

Serum protein-bound iodine¹ was determined several times before beginning treatment. Propylthiouracil² in dosage ranging from 50 mg. three times daily to 150 mg. four times daily was given to a series of patients

¹ In the present study, protein-bound blood iodine was determined throughout by Doctor A. L. Chaney, Glendale, California.

² Propylthiouracil ("Probacil") was generously provided by Doctor Stanton M. Hardy, Medical Director, Lederle Laboratories.

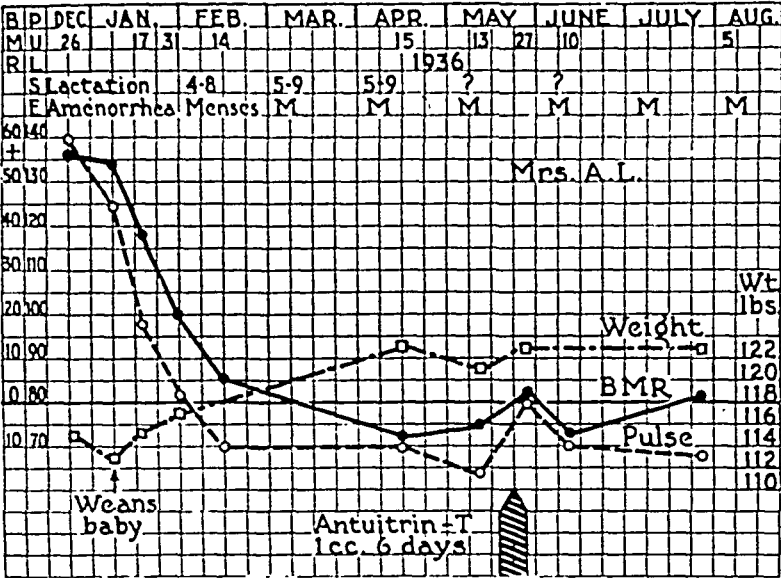


FIG. 1. (Case 1). Mrs. A. L. Example of remission of hyperthyroidism coincident with cessation of lactation and return of menstruation. (Reprinted from Starr, P., and Pomerence, H.: *Ann. Int. Med.* 15: 226-244 (Aug.) 1941. (1).

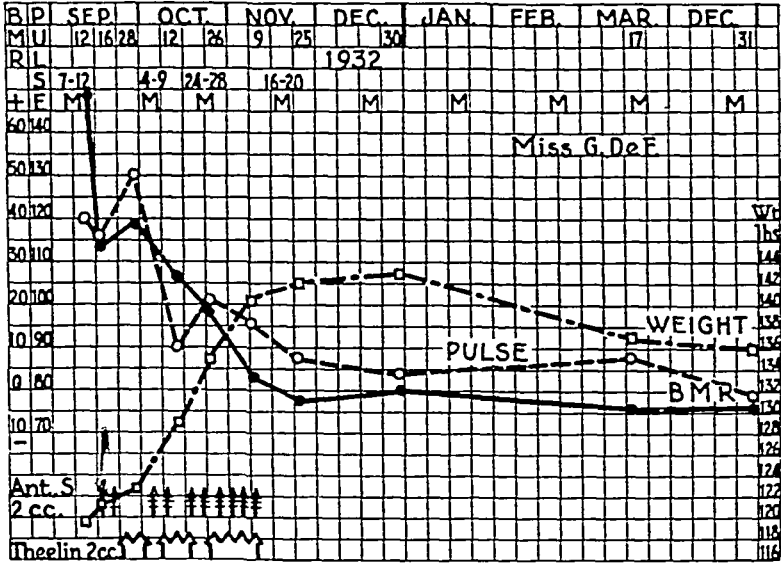


FIG. 2. (Case 2). Miss G. DeF. Example of remission of hyperthyroidism coincident with treatment with chorionic gonadotropin and theelin. (Reprinted from Starr, P., and Pomerence, H.: *Ann. Int. Med.* 15: 226-244 (Aug.) 1941. (1).

having all varieties of hyperthyroidism. During the first month, protein-bound blood iodine determinations were made at short intervals for the purpose of determining the rate of fall in proportion to the dosage given. The results of this study are shown in the following section. The drug was then continued indefinitely, patients reporting once a week for clinical estimation and differential white blood count. The progress of the treatment was followed chiefly with determinations of protein-bound blood iodine, as is shown in Figures 7, 8, and 9. After several months of normal blood iodine values and clinical control of the disease, the drug was discontinued and the reaction of the patient was followed, chemically and clinically.

CLINICAL RESULTS

Forty patients have been treated or have been under treatment since January, 1947. The summary of findings in 28 such cases is shown in Table 1. It is to be seen that in 7 of the 28, or about one-fourth of these patients, the disease was uncontrolled, *i.e.*, the protein-bound blood iodine was not within normal range nor were their clinical symptoms in abeyance. These uncontrolled patients had the following levels of protein-bound blood iodine and of propylthiouracil dosage:

Patient	Duration of therapy (months)	Daily dose (mg.)	Level of protein-bound iodine in serum (micrograms %)
J.H.	15	150	above 9 (Fig. 8)
O.H.	2	600	above 12
W.N.	4	150	above 8
P.R.	3	200	above 9
L.S.	8	300-600	above 10
M.C.	2	200	above 9
B.C.	10	300	above 9

The average duration of treatment of the entire series was six and one half months, ranging from two to fifteen months. The dosage of propylthiouracil ranged from 150 to 600 mg. daily.

Toxicity

Toxicity, as indicated by differential blood counts, developed at some time in 6 of the 40 patients, *i.e.*, 15 per cent of this small series. The white blood cell counts in these cases are shown in Table 2.

In no case was the drug discontinued because of leukopenia.

Relation between dose of propylthiouracil and protein-bound iodine in serum

Figures 4, 5 and 6 show the blood iodine in patients given 150, 300 and

TABLE 1

Patient	Propylthiouracil		Present clinical condition	Toxic reaction	Remarks
	Duration of treatment (months)	Daily dose (mg.)			
I.B.	11	200	Controlled	?	No relapse when treatment discontinued after 11 mo.
D.C.	4½	300	Controlled	0	Treatment continued.
M.C.	2	200	Uncontrolled	0	Changed to methylthiouracil after 2 mo.
B.C.	10	300	Uncontrolled	0	Treatment discontinued.
K.D.	7	150	Controlled, with PBI* below 5, for 4 mo.	0	Treatment continued.
M.D.	9	300	Controlled	0	Relapse when treatment discontinued after 9 mo.
E.D.	6	300	Controlled		Treatment continued.
E.F.	9	300	Controlled, with PBI below 5, for 2 mo.	0	Relapse when treatment discontinued after 9 mo.
G.G.	2	600	Controlled	0	Treatment continued.
L.G.	11	200	Controlled incompletely	0	Not cured.
J.H.	15	150	Uncontrolled	0	Unchanged.
O.H.	2	600	Uncontrolled		Thyroidectomy.
V.L.	9	600	Controlled	Leukopenia	Thyroidectomy.
		150	Uncontrolled		
H.L.	8	200	Controlled	0	Treatment continued.
U.M.	2	300	Uncontrolled	0	Thyroidectomy.
W.N.	4	150	Uncontrolled	?	Thyroidectomy.
B.P.	14	300	Controlled	0	Relapse when treatment discontinued after 14 mo.
E.P.	1	600	?	Leukopenia	?
P.R.	5	400	Partial control		Thyroidectomy.
	2	600	Controlled		
C.R.	10	300	Controlled	0	Treatment continued.
L.S.	8	300	Controlled	0	Thyroidectomy.
M.S.	9	200	Controlled	Leukopenia	Relapse when treatment discontinued after 9 mo.
M.D.S.	9	300	Controlled	0	Treatment continued.
L.T.	12	300	Controlled	0	Treatment continued.
D.T.	8	150	Controlled	0	Relapse when treatment discontinued after 8 mo.
R.W.	4	300	Controlled	0	Treatment continued.
T.W.	4	600	Controlled	Leukopenia	Died.
K.Y.	9	200	Controlled	Leukopenia	Relapse after treatment had been discontinued for 9 mo. Controlled when treatment started again.

* Protein-bound blood iodine

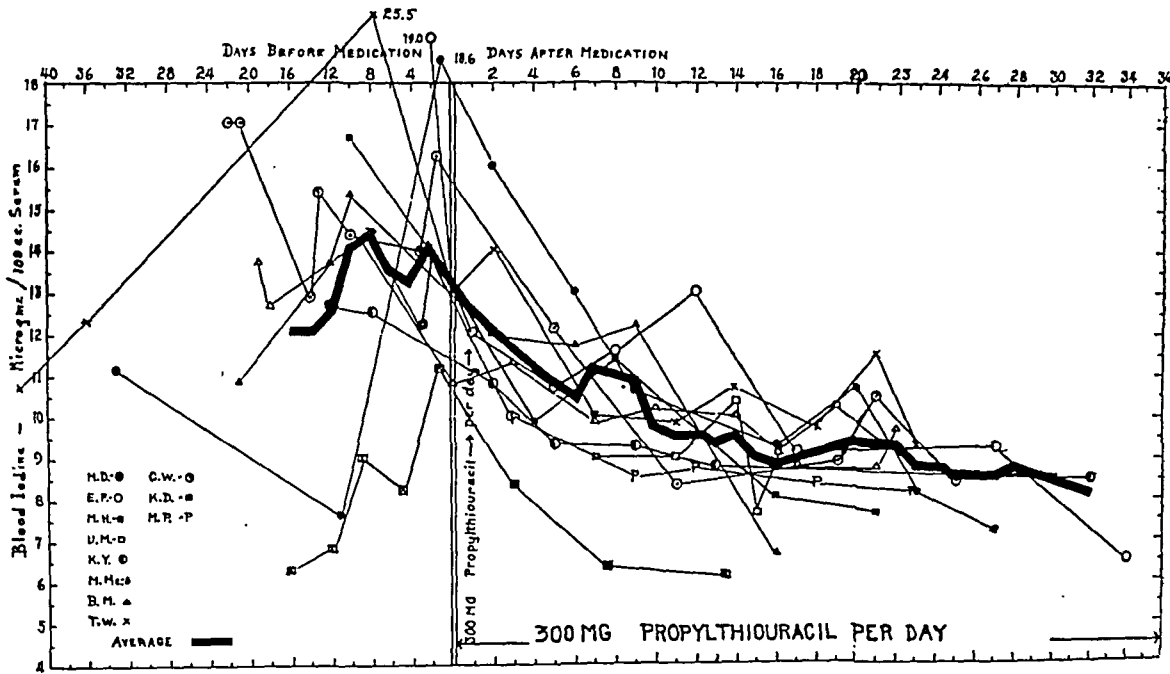


FIG. 5. Serum protein-bound iodine values in 11 patients given 300 mg. propylthiouracil per day.

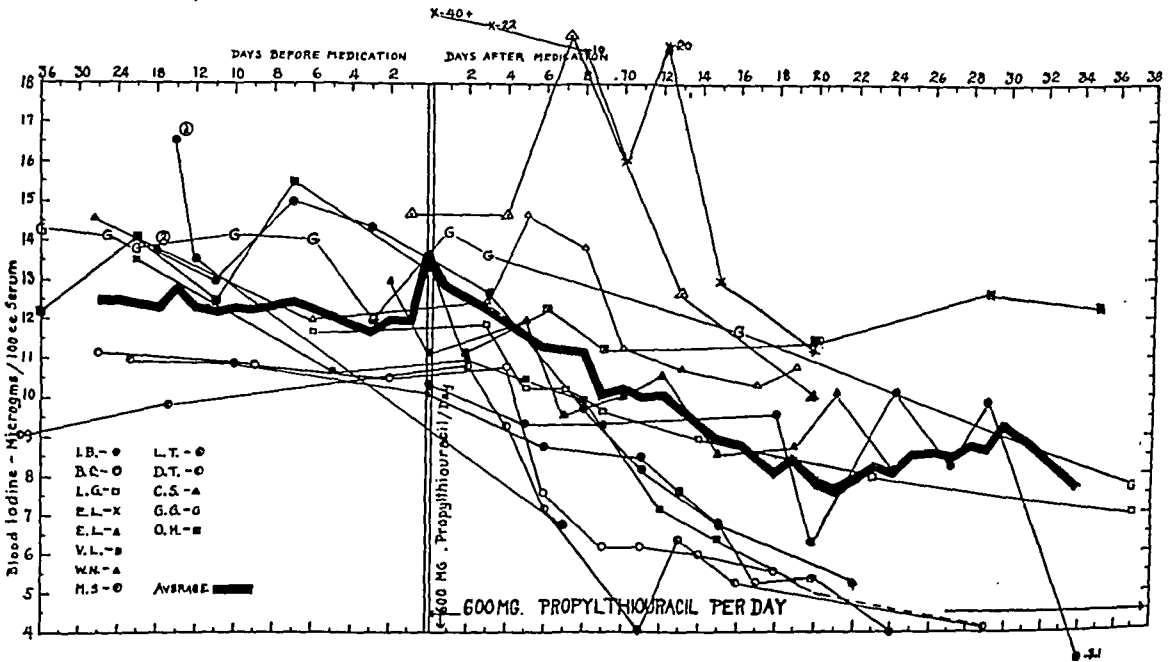


FIG. 6. Serum protein-bound iodine values in 13 patients given 600 mg. propylthiouracil per day.

TABLE 2. SIX PATIENTS SHOWING LEUKOPENIC AND GRANULOPENIC REACTION DURING PROPYLTHIOURACIL TREATMENT (FROM A TOTAL OF 40 CASES)

Patient	Duration of therapy before toxic reaction occurred	Daily dose (mg.)	W.B.C. and per cent of polymorphs		Time required for recovery	Dosage at this time (mg.)	Remarks
			before reaction	during reaction			
V.L.	3 weeks	600	5000—50%	2800—14%	—	—	Thyroidectomy
M.D.S.	7 weeks	150	9200—63%	6200—23%	8 weeks (8000—59%)	100	Dose reduced
M.S.	17 days	600	6100—59%	3200—48%	3 days (5100—68%)	150	Dose reduced
V.S.	1 week	150	6750—36%	7400—18%	4 days (7800—41%)	150	No interruption of medication
T.W.	10 days	300	5300—78%	3850—64%	8 weeks (6600—38%)	600	Dose increased to 600 mg. daily
K.Y.	2 months	150	7650—60%	5200—29%	7 weeks (7300—56%)	200	Medication not interrupted

600 mg. per day respectively. It is to be seen from the average iodine curves that there is no striking difference in the immediate reaction to the drug, although in a few instances lower levels are reached in the 600 mg. dose series. Even with 600 mg. per day, the average blood iodine of the group does not reach a normal level.

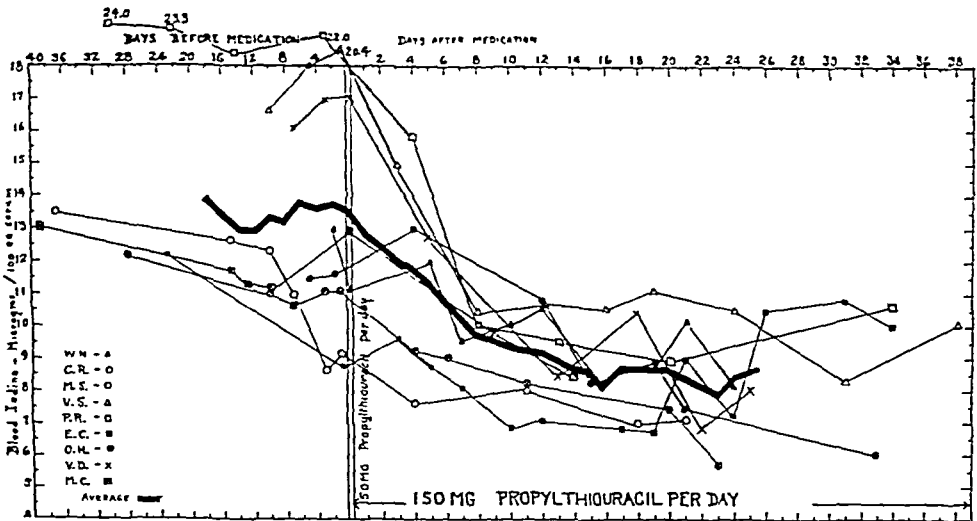


FIG. 4. Serum protein-bound iodine values in 9 patients given 150 mg. propylthiouracil per day.

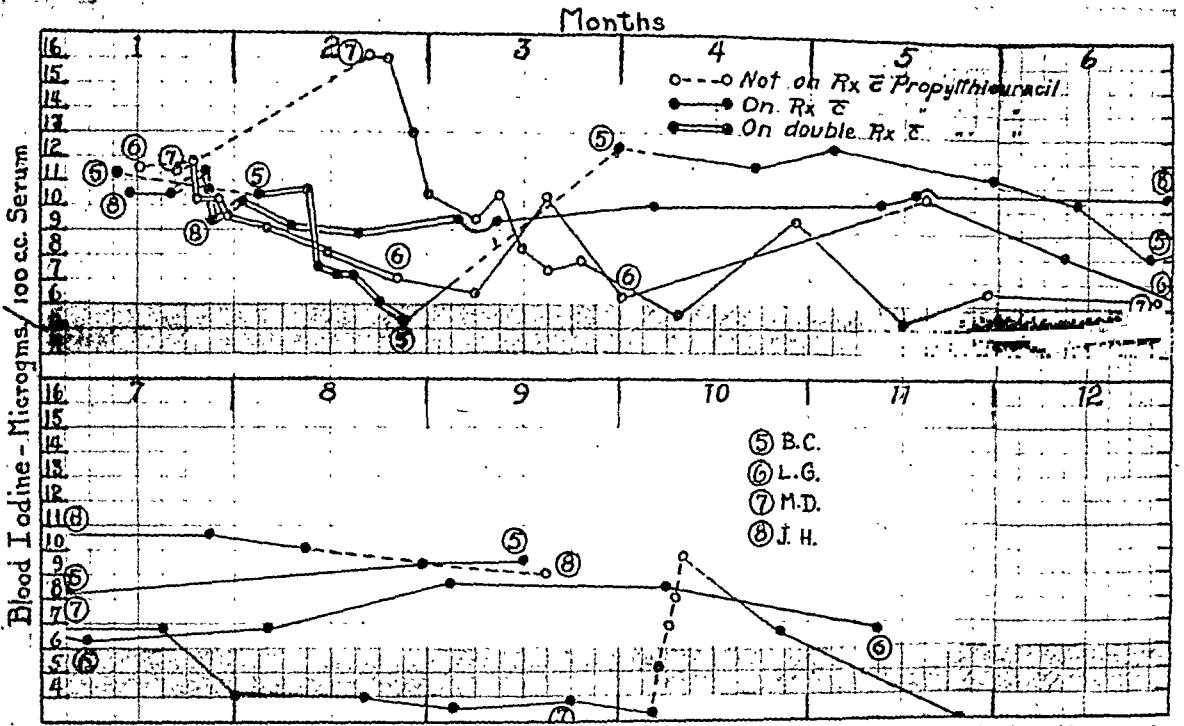


FIG. 8. Serum protein-bound iodine values in 4 patients under treatment with propylthiouracil. Note maintenance of high values in Case 8 and Case 5, normal values in Case 6, and the acute relapse in Case 7 after 9 months of successful treatment.

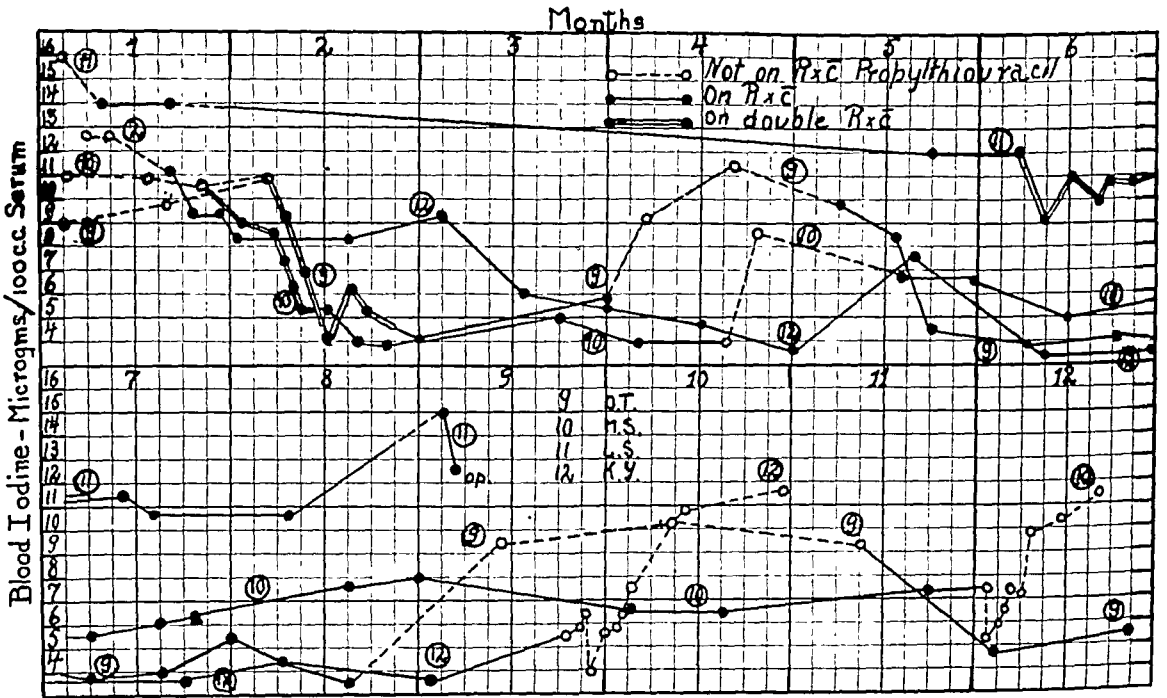


FIG. 9. Serum protein-bound iodine values in 4 patients. Note high values in case 11, in spite of large dosage. Acute relapse occurred in the remaining 3 cases, even when control had been very satisfactory for 9 months, as in Case 12.

Results of prolonged treatment

The results of prolonged treatment are shown in Figures 7, 8 and 9, in which the individual protein-bound iodine determinations of each patient are shown chronologically. It is to be seen that in a few cases continuous treatment over long periods of time did not control the protein-bound

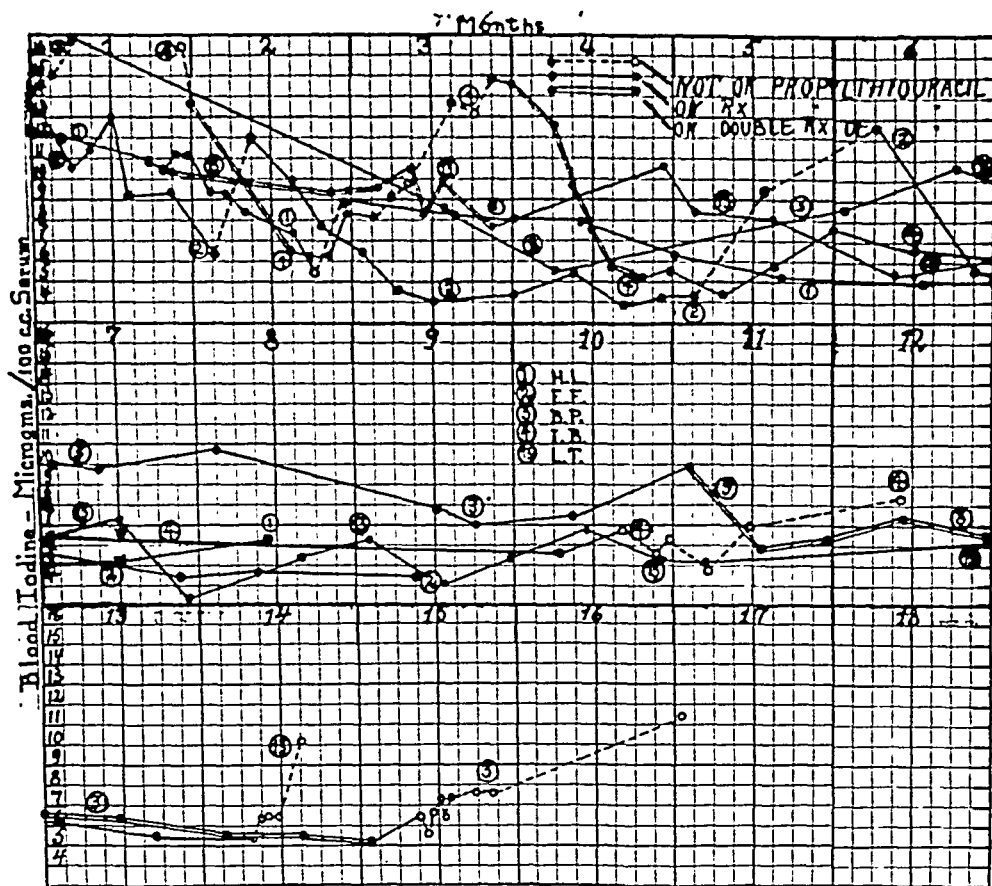


FIG. 7. Serum protein-bound iodine values in 5 patients (dotted lines indicate discontinuance of medication; single lines, usual medication of 300 mg. propylthiouracil daily; double lines, 600 mg. propylthiouracil daily). Cases 1 and 2: treatment continued to date. Case 2: relapse after 4 months of treatment. Case 3: relapse after 14 months of treatment. Case 13: relapse after 13 months of treatment. (In this and subsequent figures, "Rx c" means "treatment with").

iodine, even though the dosage was large, whereas in other cases a low level was found even though the dosage was relatively small (compare D. T. and L. S. in Fig. 9).

It is evident from a study of the individual curves in Figures 7, 8 and 9 that the daily amount of propylthiouracil required to keep the protein-

This would indicate that instead of 85 per cent, less than $\frac{1}{3}$ have been demonstrated to have a prolonged remission. Frisk (2) states that of 126 patients given prolonged treatment with methylthiouracil, a drug which is as strong as propylthiouracil, 18 patients or approximately 14 per cent were found to have a prolonged remission of at least eighteen months' duration.

If a medication will induce a prolonged, if not permanent, cure of hyperthyroidism in 85 per cent of patients with hyperthyroidism, even though a year's treatment is involved, general use of the drug for this purpose is warranted in spite of the possibility of some drug intoxication. If, on the other hand, only $\frac{1}{3}$ or fewer of the patients receive lasting benefit, it would seem that use of the drug in prolonged therapy is unwarranted.

Although the granulopenia in our patients was not severe and was not accompanied by oral or systemic signs of agranulocytosis, we feel that a drug which will cause mild bone marrow depression in so many cases must be used with caution and that it may cause much more dangerous reactions in some cases. Close clinical observation is therefore required while the drug is in use.

SUMMARY

1. Hyperthyroidism was controlled in 21 of 28 patients for from one to ten months during the administration of from 150 to 600 mg. of propylthiouracil daily.

2. The disease was not completely controlled, as indicated by abnormal serum protein-bound iodine levels in 7 of 28 patients.

3. Granulopenia, evidenced by polymorphonuclear percentages ranging from 14 to 48 per cent, was found in 6 patients out of a total of 40 patients to whom the drug was given, indicating a toxic effect on the bone marrow in 15 per cent. Medication was not interrupted in any patient because of this observed granulopenia.

4. The initial fall of protein-bound blood iodine during the first twenty-four days of treatment was not significantly greater during therapy with 600 mg. of propylthiouracil a day than with 150 mg. a day.

5. A relapse, judged by both clinical and chemical findings, occurred in nearly every case in which the drug was discontinued, even when control had been complete chemically for as long as ten months.

CONCLUSION

Propylthiouracil can be used to control hyperthyroidism.

It is frequently ineffective.

It usually will not induce a permanent remission following even prolonged use.

It may produce granulopenia in about 15 per cent of patients.

bound serum iodine at low levels varies considerably from one patient to the next. For examples, in one patient a small dose such as 100 mg. per day will keep the iodine level low (D.T., No. 9, Fig. 9), whereas in another patient 600 mg. per day will not do so (L. S., No. 11, Fig. 9).

Remissions

Permanent remissions have not been obtained when the medicine was discontinued after several months of satisfactory control. Even though the disease was apparently well suppressed and the protein-bound blood iodine was at a low level, discontinuance of the drug was followed by immediate relapse.

For example, in E.F. (No. 2, Fig. 7) the blood iodine was easily maintained below 5 micrograms per cent for two months, with immediate relapse on omission of medication. She is still taking propylthiouracil and shows a blood iodine level of 4.5 mg. per cent in the ninth month of treatment. Miss D.T. (No. 9, Fig. 9) has had a relapse twice after control of the disease, indicated by blood iodine values below 6 and below 5 micrograms per cent respectively. Miss M. S., Mrs. K. Y., Mrs. I. B., Mrs. B. P., Miss M.D., and Mrs. L.T. have each had relapses after 4½, 5, 6, 6, 6 and 8 months of normal blood iodine findings. Thus, every patient in this series has had a relapse on discontinuance of the medication (Figs. 7, 8 and 9) with the possible exception of I. B. (Fig. 7).

DISCUSSION

Our experience with propylthiouracil would seem to show that the amount of circulating thyroid hormone, as represented roughly by the protein-bound serum iodine, can be reduced in most cases of hyperthyroidism. The use of the drug, therefore, in preparation for a secondary destructive procedure to interrupt the disease (thyroidectomy), is justified. Its use as a prolonged treatment in the hope that the disease will be cured or that an indefinite remission will be induced seems unjustified from this study.

The proportion of cases in which a prolonged remission or cure can be induced by propylthiouracil is extremely important. Different authors report different results. E. B. Astwood (personal communication) finds that 85 per cent of his patients have lasting remissions after discontinuing propylthiouracil. He emphasizes that "a state of hypothyroidism must be maintained for some months in order to obtain this result." Williams (3), reporting on thiouracil, a weaker antithyroid drug than propylthiouracil, states that "of 111 thyrotoxic patients who were treated with thiouracil and had a cessation of therapy, 51 are in remissions that have lasted for 3 to 31 months . . . 33 have remained well for more than eighteen months."

THE USE OF TRACER DOSES OF RADIOACTIVE IODINE, I^{131} , IN THE STUDY OF NORMAL AND DISORDERED THYROID FUNCTION IN MAN*†

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THIS report presents the results of a fact-finding survey concerning the uptake by the thyroid of tracer doses of radioactive iodine of mass 131, eight day half-life, during normal and disordered thyroid gland function in man. This type of study is made possible by the inability of the animal and human organisms to differentiate between the several isotopes of iodine. Thus the radioactive isotopes of iodine are concentrated in, and leave the thyroid gland, in the same manner as the stable form (1, 2). The natural disintegration of the radioactive material produces both gamma and beta rays (3). The beta rays are absorbed within 2 millimeters of tissue. The gamma rays pass through the skin and can be detected readily by a Geiger counter, thus providing a means of determining the presence of the isotope and its actual concentration, and serving as an indicator of the behavior of stable iodine within the body. The highly localized radiation delivered by the beta rays, and the lesser contribution of the gamma rays, have been taken advantage of for therapeutic purposes (4, 5, 6, 7).

In the latter half of 1946, the United States Atomic Energy Commission released for investigative purposes, radioactive iodine of mass 131 (eight day half-life) from the atomic pile. The material became available to the authors in October 1946 and was used to assess its value in the therapy and diagnosis of toxic goiter. The latter part of the study was soon extended to a variety of clinical conditions in an attempt to evaluate the usefulness of this particular activity of the thyroid gland, iodine uptake, as an index of over-all thyroid function.

METHODS

Radioactive iodine, I^{131} , obtained from the Clinton Laboratories, Oak Ridge, Tennessee, was used throughout the study. Activity was deter-

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widely, though the majority were within the range of plus 15 to minus 15 per cent and all patients were euthyroid clinically. The radioactive iodine uptake of the thyroid at 24 hours has a range of 7 to 49 per cent of the tracer dose. If a normal range of uptake is arbitrarily set with an upper limit of 35 per cent, 51 of the 57 cases, or 91 per cent are included. Six of the 57 cases, or 9 per cent are found above this range. If the upper limit is set at 40 per cent uptake, 2 in 57, or only 4 per cent of the cases exceed this point. The values exceeding the normal limits overlap with the lower levels of uptake found in toxic goiter, but the overlap is less for the 35 per cent limit, as will be seen subsequently.

TABLE 2. RADIOIODINE UPTAKE IN HYPERTHYROIDISM

Per cent uptake at 24 hrs.	Toxic diffuse goiter			Toxic nodular goiter
	Primary	Recurrent after treatment by		
		I ¹³¹	Surgery	
50-	48	9		7
40-49	9	5	8	
35-39	2	2	1	
30-34	1		1	
20-29	1	1		
10-19		2		
0-9				

In this group the effect of age is significant. One instance of uptake less than 10 per cent was noted in a normal subject aged 72. All but one of the uptakes in the lowest normal range of 10 to 15 per cent uptake, and the one uptake below 10 per cent just mentioned, occurred in women 40 years of age or more.

B. In hyperthyroidism.

1. *Patients with active hyperthyroidism.* A group of 97 cases of toxic goiter has been studied (Table 2). These have been divided into toxic diffuse and toxic nodular goiter. The former group has been subdivided into primary, or untreated cases and those that were recurrent despite therapy by either operation or radioactive iodine. A similar range of uptake is noted in all groups except in the one in which hyperthyroidism recurred after treatment with radioactive iodine. For the toxic group as a whole, 91

mined on receipt of the material, by means of a Geiger counter standardized according to the millicurie established by Marinelli from ionization chamber measurements at the Memorial Hospital (8). Variable standardization procedures are in use in different laboratories so that discrepancies of the order of 100 per cent may occur in estimating a millicurie of radioactive material and this factor must be taken into account in comparing the reports of different institutions. Tracer doses of I^{131} without significant amounts of stable iodine (carrier) were given by mouth in the nonfasting state. Doses of between 40 and 100 microcuries were used, the smaller

TABLE 1. RADIOIODINE UPTAKE IN EUTHYROIDISM

Per cent uptake at 24 hrs.	Control subjects
50-	—
40-49	2
35-39	4
30-34	8
20-29	24
10-19	18
0- 9	1

amount given being routinely in the later studies. Radioactivity in the thyroid gland was measured with a Geiger counter placed at a standard distance of 15 centimeters from the neck, with the thyroid isthmus as a center, and with the head and neck in a special holder. A wide counter aperture was designed which permits the accurate measurement of radiation from an area of the neck measuring 15×10 cm., sufficient for all except extremely large goiters. Measurements of uptake were made at the end of a 24-hour period. All patients were studied after complete medical work-up or after operation. No treatment was given prior to testing, except when mentioned.

RESULTS¹

A. In control patients with euthyroidism.

Fifty-seven ward and clinic subjects with disorders not known to affect the endocrine system were studied (Table 1). Ages ranged from 16 to 72 years. There were 23 men and 34 women. Basal metabolic rates varied

¹ At present, the series includes about twice as many patients as when this article was submitted for publication. The observations reported in the various conditions are the same except for a larger number in each category.

TABLE 4. RADIOIODINE UPTAKE IN REMISSION OF HYPERTHYROIDISM PRODUCED AFTER THERAPY

Per cent uptake at 24 hrs.	Surgery	Propyl-thiouracil	X-ray	I ¹³¹
50-40-49 35-39	1	1 1	1	4
30-34 20-29 10-19	1	1		3 4 4
0-9				

radiation with radioiodine. It is clear that despite the return of the metabolic rate to normal, the iodine uptake often continues at an elevated level. Eight of the 21 cases in the series, or 38 per cent, have values in excess of the 35 per cent upper limit of normal uptake.

C. In miscellaneous thyroid disorders.

A variety of thyroid disorders other than toxic goiter have been surveyed by the tracer technique (Table 5). There are 44 cases in this group. The basal metabolic rates of these patients are normal, except for 1 instance of chronic thyroiditis and 3 of hypothyroidism.

1. *Nontoxic goiter.* The 3 patients with nontoxic diffuse goiter and 8 patients with nontoxic nodular goiter had normal basal metabolic rates

TABLE 5. RADIOIODINE UPTAKE IN VARIOUS THYROID DISORDERS

Per cent uptake at 24 hrs.	Nontoxic goiter		Malignant exophthalmos	Thyroiditis		Carcinoma	Hypothyroidism
	diffuse	nodular		acute	chronic		
50-40-49 35-39	1 1	1 1	3* 1		1* 1 1		
30-34 20-29 10-19		2 3 1	3 1		2 1 5	2 5	
0-9	1			6	1	2	3

* 1 patient thyrotoxic.

of the 97, or 94 per cent of the cases have uptakes exceeding 35 per cent. Six of the 97, or 6 per cent show uptakes not in excess of the normal range. In the primary group, only 2 cases, or 3 per cent, are within normal limits, whereas in the cases recurrent after radioactive iodine therapy, the incidence of uptake within the normal or even subnormal range is about five times as great (16 per cent). If the upper limit of normal is considered

TABLE 3. CORRELATION BETWEEN EXTREMES OF RADIOIODINE UPTAKE AND BASAL METABOLIC RATE IN EUTHYROIDISM AND HYPERTHYROIDISM

Thyroid status	Patient	B.M.R. %	Per cent uptake at 24 hrs.
Euthyroid	Me.	+23	Greater than 35%
	O'to.	+ 3	49
	Pe.	+ 8	43
	Ke.	+15	43
	Fa.	-16	39
	Sa.	+20	39
	Ol.	+53*	36
Hyperthyroid			Less than 35%
	Sp.	+33	13
	Re.	+20	15
	Bl.	+50	19
	Ct.	+76	23
	Ma.	+24	34

* Could not obtain true B.M.R.

as 40 per cent uptake in order to include practically all control values, the number of toxic goiter cases falling within normal limits is increased to 11 in 97, or 11 per cent. There is, therefore, a significant and unavoidable overlap between control and hyperthyroid patients. A normal range with an upper limit of 35 per cent uptake permits 9 per cent of normal cases to be in the toxic range and 6 per cent of all toxic cases to be in the normal range (Tables 1, 2 and 3); whereas an upper limit of less than 40 per cent uptake alters these figures to 4 and 11 respectively. The individual data for the overlapping group, including basal metabolic rates, are presented in Table 3. The tables in this paper are divided to indicate a normal range of 10 to 35 per cent uptake.

2. *Patients with remission of hyperthyroidism.* The effect on radioactive iodine uptake of successful therapy for toxic goiter has been studied in 21 patients (Table 4). Remission followed either operation, cessation of antithyroid drug administration, external radiation with x-rays or internal

TABLE 6. RADIOIODINE UPTAKE IN PITUITARY AND ADRENAL DISORDERS

Per cent uptake at 24 hrs.	Pituitary tumors		Adrenal disorders	
	eosinophile	chromophobe	Addison's disease	Cushing's syndrome
50-40-49	2			
35-39	1			
30-34	1		1	
20-29	2	1	1	
10-19	4	5	3	1
0-9		3		1

the low basal metabolic rate regularly found in this condition. The distribution of uptake is preponderantly in the low normal range. The uptake in the 2 instances of Cushing's disease, presumably, with adrenal cortical hyperfunction, is unusually low.

E. In nutritional disorders.

Eight patients with fairly extreme degrees of obesity and normal basal metabolic rates and 6 patients with anorexia nervosa (5 with low basal metabolisms of minus 21 to minus 30 per cent), were examined (Table 7). The uptake in all was within normal limits except for 1 instance of obesity. The lack of correlation between basal metabolic rate and uptake of I¹³¹ is emphasized in the anorexia nervosa group. The obese patient with the low uptake had diarrhea at the time of the test and may have failed to absorb much of the radioactive iodine from the gut.

TABLE 7. RADIOIODINE UPTAKE IN NUTRITIONAL DISTURBANCES

Per cent uptake at 24 hrs.	Obesity	Anorexia nervosa
40-49		
35-39		1
30-34	3	1
20-29	3	1
10-19	1	3
0-9	1	

and were clinically euthyroid. The same approximate distribution of uptake as in the control subjects was noted.

2. *Malignant exophthalmos.* This group consists of 8 instances of proven, or clinically suspected, malignant exophthalmos—7 patients in which the exophthalmos was present without a rise in basal metabolic rate, and 1 patient with an elevated basal metabolism. The radioiodine uptake of 2 of the 8 patients with normal metabolic rates exceeded the highest value for any control subject, and there is one other uptake greater than 40 per cent. The patient with definite thyroid overactivity also had a considerably elevated uptake.

3. *Acute thyroiditis.* The 6 cases in this group are of interest in view of the extremely low uptake of radioactive iodine in every instance, despite a normal basal metabolic rate. Follow-up measurements are not available in all, but there is a lag of one or more months until recovery of the uptake function occurs.

4. *Chronic thyroiditis.* The low uptake of iodine shown in acute thyroiditis is found in only 1 of the 7 chronic cases. The cases of Riedl's or of Hashimoto's struma have been combined in the table. A very low uptake may have diagnostic significance in differentiating these cases from carcinoma, providing the effect of age is also taken into account. More data are needed.

5. *Carcinoma of the thyroid.* Nine patients with untreated malignancy of the thyroid showed normal uptakes of iodine. In none of these had carcinoma entirely replaced the normal gland. Clinical information is missing in the 2 instances with uptakes of less than 10 per cent but these are suspected of having received previous therapy of some sort.

6. *Hypothyroidism.* The known low uptake of iodine in hypothyroidism (7) is confirmed in the 3 cases tested.

D. In pituitary and adrenal disorders

Nineteen examples of either eosinophile or chromophobe tumor of the anterior hypophysis have been studied (Table 6). The range of iodine uptake in the acromegalic group follows the normal pattern despite general but slight elevation of basal metabolic rate. In the chromophobe group the values hug the lower limits of normal or are below. Four outspoken and untreated instances of panhypopituitarism occur in this latter group. These patients had basal metabolic rates ranging from minus 17 to minus 41 per cent and levels of serum cholesterol (fasting) which ranged from 260 to 372 mg. per cent. However, 3 of the 4 showed uptake of radioactive iodine within the lower normal range.

Five instances of fairly advanced underfunction of the adrenal cortex *i.e.*, Addison's disease, were examined with the tracer technic in view of

TABLE 8. EFFECT OF PRECEDING ADMINISTRATION OF DESICCATED THYROID OR OF STABLE IODINE ON RADIOIODINE UPTAKE

Desiccated thyroid group					
Patient	Daily dose Gm.	Weeks since thyroid omitted	B.M.R. %	Cholesterol	Per cent uptake at 24 hrs.
C.B.	0.3	0	+ 5	150	4
		8	-15	204	26
P.O.B.	0.24	0	+ 6	120	2
		7	-17	168	24
F.W.	0.12	0	-12	195	4
		6	-13	201	3
R.B.	not known	10	+ 1	184	4
Stable iodine group					
Patient	Daily dose Gm.	Weeks since iodine omitted	B.M.R. %	Cholesterol	Per cent uptake at 24 hrs.
M.F.	iodide by mouth	1½	+69	195	2
		7			2
R.F.	iodide by mouth	4	+23	208	8
S.M.	iodide by mouth	4	-12	197	46
LS.	iodide by mouth	12	+45	288	0
M.C.	Diodrast	½	± 0		10
J.S.	I.V. pyelogram	2			3
		4			4

throughout the body. Mathematical treatment of the data from urine studies may well reflect gland uptake. However, apart from the inherent possibility of inaccuracy from indirect methods, the danger of mistakes in the collection of urine, known or unknown to the physician, tends to rule out this method for routine clinical use.

There is little doubt at present that accurate direct measurement of the

F. In miscellaneous endocrine disorders.

Single examples of several endocrine disorders other than those listed above have been tested in pilot experiments. No significant deviation from normal uptake was noted.

Effect of preceding administration of desiccated thyroid or of stable iodine

The tracer uptakes by the thyroid gland of 4 euthyroid patients, during and after the administration of desiccated thyroid, are presented in Table S. The striking depression of uptake during therapy and the occasional long delay in recovery after discontinuance are clearly shown. As little as 0.12 Gm. of desiccated thyroid by mouth may cause a depression of uptake to 4 per cent of the tracer dose, *i.e.*, to the hypothyroid range. In the case in which uptake failed to return to normal within ten weeks after omission of the drug, the patient denied its resumption and there was no reason to suspect her veracity.

Six cases previously given stable iodine were studied at varying intervals after the last administration of the drug. The first 4 patients listed had received sodium or potassium iodide for several weeks to several months; patient M.C. had had a single intravenous injection of Diodrast to outline an obstructed brachial vein, and patient J. S. had been given diiodophenolphthalein, for an intravenous pyelogram. The known depression of uptake after iodine administration is confirmed (9). Recovery is seen to be delayed in all but S.M. (in whom uptake was high in the fourth week off iodine) and in M.C. where only four days had elapsed to the time of the tracer test. In one patient, M.F., recovery is still greatly delayed seven weeks after cessation of therapy. Again, there is no reason to doubt the reliability of the subject in his statement that he had had no further iodine. The data in patient L. S. are complicated by the fact that propylthiouracil was taken intermittently during the twelve weeks since omission of iodine. This medication was stopped four weeks before the tracer test, which reveals a zero uptake.

DISCUSSION AND CONCLUSIONS

A fact-finding survey of the uptake of tracer doses of radioactive iodine I^{131} , has been made in conditions of normal and disordered thyroid function in man. Direct measurement of the uptake by the thyroid gland has been determined as described in a previous publication (6). This method was decided upon as more accurate and feasible than the determination of the urinary excretion of radioactive iodine. There is a reciprocal relationship between uptake by the gland and excretion in the urine (10). The sum of uptake by the thyroid gland and excretion in the urine accounts for from 60 to 80 per cent of the administered iodine, the rest being distributed

ministration of thyroid substance containing stable iodine, there may be a distinct discrepancy between iodine uptake and basal metabolic rate. In these patients, the basal metabolic rate appears to be markedly altered without detectable change in thyroid function as indicated by radioiodine uptake. Explanation for this may be found in one of several hypotheses: in an inherent ability of tissues to change their rate of metabolic activity in the face of a constant thyroid hormone blood level; in a dissociation between inorganic iodine uptake and a subsequent hormone release by the gland, such as is seen with antithyroid drug administration; or in present inability to interpret the significance of minor, or temporary, degrees of change in iodine uptake and in plasma bound iodine, especially since there is generally no preceding baseline of uptake to cover the period when the individual was healthy, or no adequate history in respect to possible preceding iodine intake such as with iodized salt or medicinal iodides. Further study will be necessary before one of these, or another reason can be accepted.

The effect of large doses of desiccated thyroid in causing a reduction of thyroid activity is now well known (14, 15). The minimal dose of desiccated thyroid which will accomplish this result and the fact that almost complete suppression of uptake may exist for weeks after cessation of therapy in some people, is not so well appreciated. The extensive use of desiccated thyroid by the gynecologist for the treatment of menstrual and sterility difficulties, and by many other physicians for the treatment of obesity, should perhaps be subjected to consideration from this standpoint. The occasional low plasma iodine levels in obesity reported by Williams (16) may be possibly explained by the use of thyroid at some period shortly before the determinations.

Stable iodine too, has been recognized as capable of reducing iodine uptake by the gland. Again, the long duration of the suppression response and the discrepancy from the normal basal metabolic rate are brought out in this study and are of importance from the standpoint of mechanisms involved and in the clinical application of the tracer test.

From the findings we have demonstrated, it can be seen that the radioactive iodine tracer test, when used as a clinical tool for determination of thyroid function, has diagnostic usefulness only when the results are properly interpreted in conjunction with other clinical and laboratory aids. It has not replaced sound clinical judgment.

SUMMARY

1. The uptake of a tracer dose of I^{131} by the thyroid gland has been measured by means of a Geiger counter, directly over and 15 centimeters

iodine uptake by the gland is feasible (6). Errors in determination may result when the dimensions of the gland exceed those measurable by a given Geiger counter aperture. A large counter with a wide aperture at some distance from the neck can be designed to cover an area wide enough for almost every type of gland. Such an arrangement is not useful for localization within, or measurement of uptake in part of the gland. A small counter placed directly against the neck is suitable for this latter purpose but is subject to error if used for over-all uptake measurement. By means of the Geiger counter, iodine uptake by the gland can be expressed in terms of the percentage of a tracer dose collected at a given time interval or as the rate of accumulation during a given period (11).

The present study is based on the determination of the percentage uptake of the radioiodine at 24 hours after ingestion, at which point uptake has become stabilized (6) and significant excretion has not begun except in rare instances of toxic goiter. Only one determination is necessary and the method is convenient for routine clinical practice.

The diagnostic usefulness of this technic has needed documentation. In the controls studied, a range of uptake of between 10 and 35 per cent of the tracer dose at 24 hours has been found to include all but 9 per cent of cases. The significance of such limits of normal uptake requires evaluation.

Most of the low values between 10 and 15 per cent, and a single value of below 10 per cent were, found in women over 40 years of age. This physiologic decrease in uptake has been found by Perlmutter (12) in both sexes, accompanied by normal plasma precipitable iodine levels. Thus, the proposition of decreased thyroid function based on low tracer uptake values must take into account the effect of aging.

High uptake values at the upper range of normal or above also require interpretation. A 35 or 40 per cent uptake limit permits an appreciable percentage of cases of proven toxic goiter to be included in the normal range. This same overlap is found when the "accumulation gradient" is determined and occurs with plasma precipitable iodine and basal metabolic rate determinations. A more precise method may have been found in the more recent technic proposed by Stanley and Astwood (13), although it has as yet been applied only to the study of the effectiveness of antithyroid drugs, and has not been used to determine whether it will narrow the percentage overlap between normal and toxic goiter patients.

The degree of correlation of radioiodine uptake with the basal metabolic rate in conditions other than toxic goiter is also of great interest. While unquestionably there is a general relationship, lack of correlation is not infrequent. Thus, after unsuccessful or successful therapy for toxic goiter in malignant exophthalmos, acute and occasionally chronic thyroiditis, pituitary and adrenal disorders, anorexia nervosa, and following the ad-

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away from the gland, in normal subjects and in patients in whom a knowledge of thyroid function might be of interest.

2. The normal range of uptake is between 10 per cent and 35-40 per cent of the administered dose, at 24 hours.

3. There is a small but significant overlap of uptake between the control subjects and patients with toxic goiter.

4. In cases of toxic goiter, the general correlation which exists between iodine uptake by the thyroid gland, and hormone secretion as reflected by the basal metabolic rate, can be disrupted by the treatment of the thyrotoxicosis.

5. Lack of correlation between iodine uptake and basal metabolism may be found in active thyroiditis, malignant exophthalmos, Simmonds' disease, Addison's disease and anorexia nervosa.

6. The depression of iodine uptake by previously administered thyroid substance or stable iodine may persist for weeks.

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gonadotropin, will produce complete spermatogenesis (2).

3. *A suitably concentrated source of FSH.*

In studying over 100 infertile men with the view of determining whether prerequisites 1 and 2 were met, it was found that the majority were eliminated because of 1) irreparable testicular damage, and/or 2) elevation of gonadotropins. In the current investigation, 7 infertile, but otherwise normal men were chosen who had either normal or low urinary gonado-

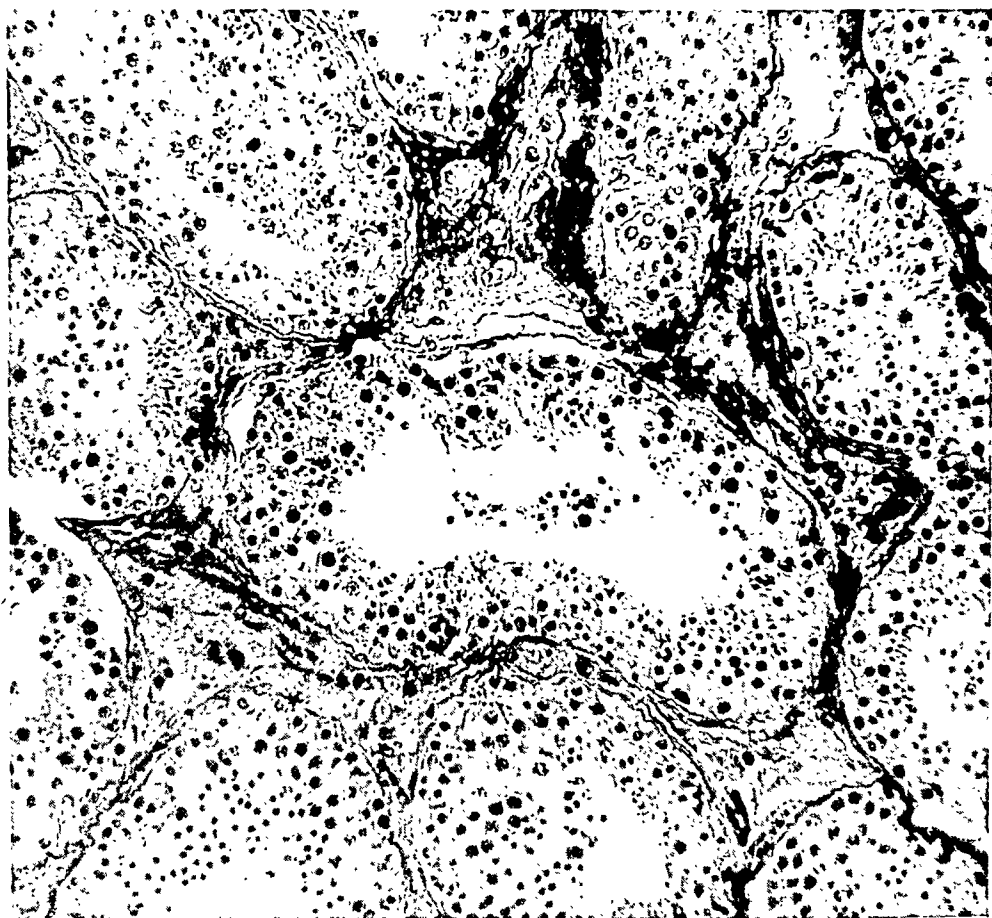


FIG. 1a. Biopsy specimen of testis from patient K. H. *before therapy*, showing seminiferous tubules of approximately normal size containing all stages of spermatogenesis, but a relative preponderance of the more immature stages, and sloughing of immature forms into the lumen of the tubules. There is a cluster of normal-appearing Leydig cells near top center. Note lack of any irreversible changes such as severe or complete hyalinization of the basement membrane. $\times 175$.

tropin excretion and in whom biopsies of the testis revealed minor, but definite alterations in spermatogenesis. The third prerequisite was met when a potent and partially purified hypophyseal extract from sheep, containing predominantly FSH, was made available to us.¹ Details concerning

¹ Generously supplied by the Schering Corporation through the courtesy of Dr. Edward Henderson.

ANTI-HORMONE FORMATION COMPLICATING PITUITARY GONADOTROPIN THERAPY IN INFERTILE MEN

II. EFFECT ON NUMBER OF SPERM, MORPHOLOGY OF THE TESTIS AND URINARY GONADOTROPINS*

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THE search for therapeutic agents effective in male infertility has been relatively fruitless to date. In recent years, hormonal therapy has been considered. Of the various hormones, the ones most likely to succeed would seem to be those that ordinarily stimulate spermatogenesis: the gonadotropins. Of the gonadotropins (from anterior pituitary, pregnancy urine and pregnant mare serum sources), one hormone that is known specifically to stimulate spermatogenesis is follicle-stimulating hormone (FSH) of the anterior pituitary (1).

It cannot be expected that FSH will prove effective in all types of male sterility. Several prerequisites are necessary before successful results can reasonably be expected. In addition to the obvious prerequisites, such as patent vas deferens, and the knowledge that the wife is potentially capable of conceiving, we have considered the following conditions to be essential:

1. *Testes with potentially reversible defects.* In many cases of infertility, the testes are irreparably damaged and attempts at therapy of any kind are useless.

2. *Gonadotropin production that is not already elevated.* If there were already an increase in endogenous gonadotropic hormones, adding FSH from an exogenous source could not be expected to stimulate spermatogenesis.

Hypogonadotropic eunuchoid men are good examples of patients meeting both these requirements, for they have infantile testes capable of responding to gonadotropic stimulation, and *subnormal* gonadotropin titers. Administration of FSH to such patients, following treatment with chorionic

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included because he was found to have subnormal titers of urinary gonadotropins.) The principal defects encountered were disorganization of spermatogenesis, lack of maturation, and sloughing of immature germinal cells into the lumen of the seminiferous tubules. There was a lack of irreversible changes such as severe or complete hyalinization of the basement membrane of the seminiferous tubules or lack of all germinal cells. The

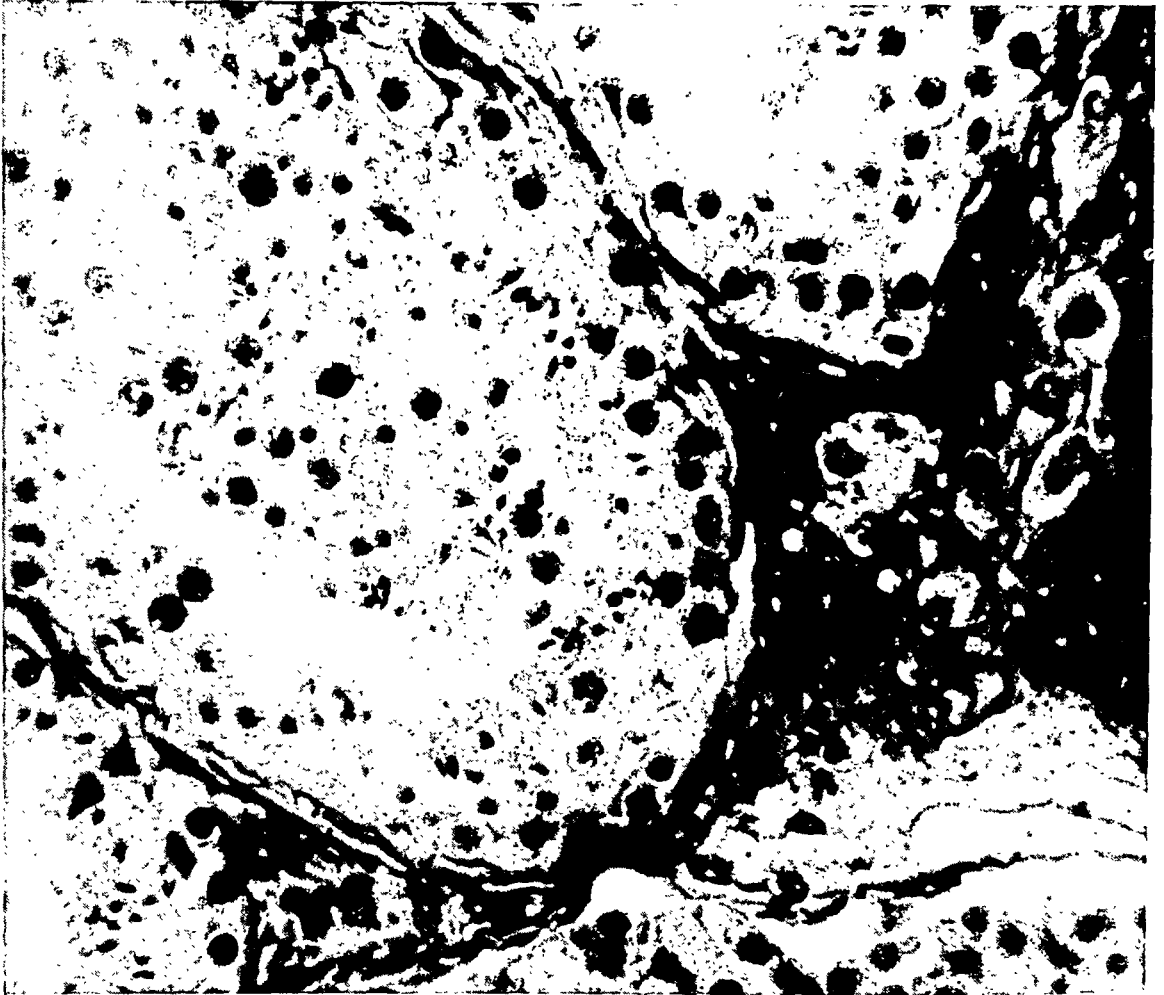


FIG. 2a. Biopsy specimen of testis from patient K. H. *before therapy*: upper left area of Figure 1a, $\times 450$. Note that all stages of spermatogenesis are represented and that normal-appearing Leydig cells are present.

encouraging feature was the presence of germinal cells in various stages of maturation which seemingly could go on to sperm formation with the proper impetus. The Leydig cells appeared normal, which was in accord with lack of any clinical evidence of androgen deficiency. The biopsies showed a marked similarity; a representative biopsy specimen (patient K. H.) is illustrated in Figures 1a and 2a.

Sperm counts. Sperm counts were performed by diluting seminal fluid

treatment are presented graphically in Figures 3 to 8, and are recorded in Table 2 of the preceding report (3).

In the preceding report (3), it was demonstrated that the administration of sheep FSH elicited formation of antihormones that were capable of neutralizing the patients' endogenous gonadotropic hormones. It is the purpose of this communication to correlate the effects of sheep FSH and

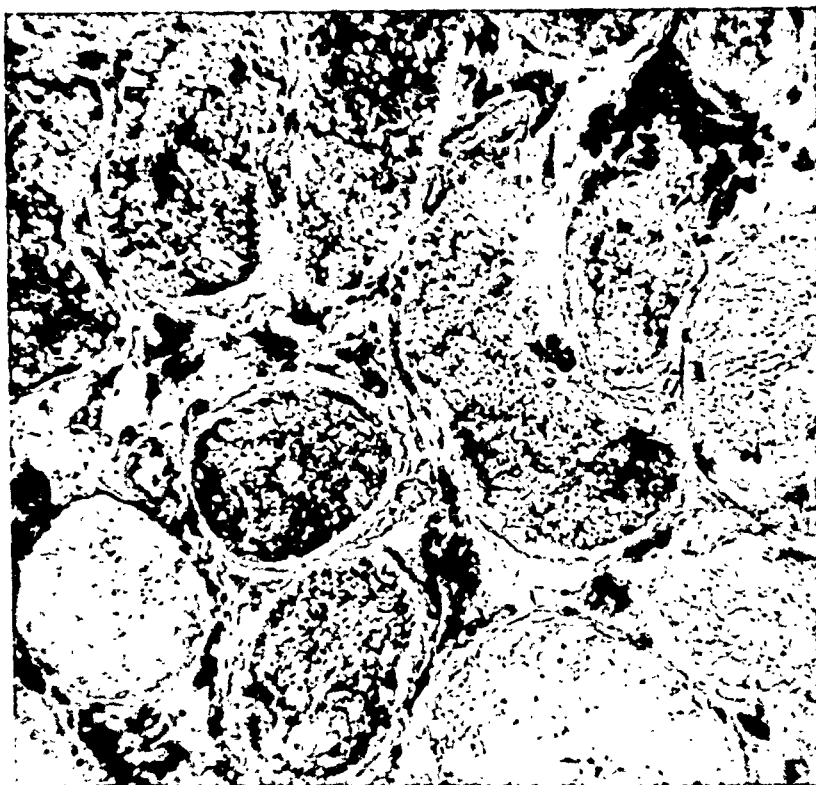


FIG. 1b. Biopsy specimen of testis from K. H. during antihormone formation. There is a striking decrease in the size of the seminiferous tubules as compared with the specimen obtained before treatment. Mature germinal cells are scarce, leaving a preponderance of spermatogonia and primary spermatocytes. Tubules are completely filled by immature cells sloughing into the lumens. Spermatozoa are present; however, these are deep-staining and seemingly are old, retained sperm. Note the lack of any recognizable Leydig cells. The appearance is similar to that occurring in pituitary failure in the human adult or in hypophysectomized animals. $\times 175$.

subsequent antihormone formation on spermatogenesis and endogenous gonadotropin excretion.

RESULTS

Morphology of the testes. Biopsy specimens of the testis were obtained from 6 of the 7 men before initiating therapy. (The seventh patient was

and after instituting therapy. Averages before, during and after therapy, however, indicated an upward trend during FSH therapy in the 6 patients, followed by a decrease during the time of maximal antihormone formation in 3 of the patients with high antihormone titers. The decrease in each instance was below the pretreatment average number of sperm. A fourth patient with similarly high antihormone titers also had a decrease in number of sperm during the time antihormones were elevated, but the onset of the decrease was delayed until two and one half months after initial antihormone detection. The 2 patients in whom numbers of sperm did not decrease experienced the least amount and shortest duration of antihormone formation.

Following the disappearance of detectable antihormones in the plasma, the average number of sperm rose to pretreatment levels or higher in the 3 cases in which a decrease had occurred, and remained at the pretreatment level or higher in the 2 in which no decrease was encountered. The patient with the delayed decrease continued to have a low sperm count, and continued to have antihormones in the plasma at the last date tested, 283 days after stopping therapy.

Urinary gonadotropin excretion:

Before treatment was instituted, gonadotropin titers were normal in 5 patients, below normal in 1 and at the upper limits of normal in 1 (Table 1). Figures for normal individuals are presented in a previous publication (4).

During FSH administration (5 patients tested) no increase in gonadotropin excretion was noted. The titers fell below the pretreatment level in 4 patients and remained unchanged in 1 (Table 1).

After FSH administration had been stopped, and during the time of antihormone formation, gonadotropin excretion was increased to the pretreatment level in 6 patients and was slightly above the pretreatment level in the remaining 1 (Table 1).

After or at about the time of disappearance of antihormones from the circulation, gonadotropin excretion was above the pretreatment level in 3 patients and equal to the pretreatment level in 3 others (Table 1).

DISCUSSION

Sperm Counts. Following the initiation of FSH therapy, there was an initial rise in the sperm output that soon reached a plateau, or sharply declined to levels that were in some instances lower than before therapy was begun. With the exception of 1 patient, the rise in output of sperm was slight. There was no improvement in the impaired motility or the abnormal

in a red or white cell pipette with dilute aqueous methylene blue, and then counting the sperm in a Neubauer counting chamber. Sperm counts are presented graphically in Figures 3 to 8. (As no more than an occasional

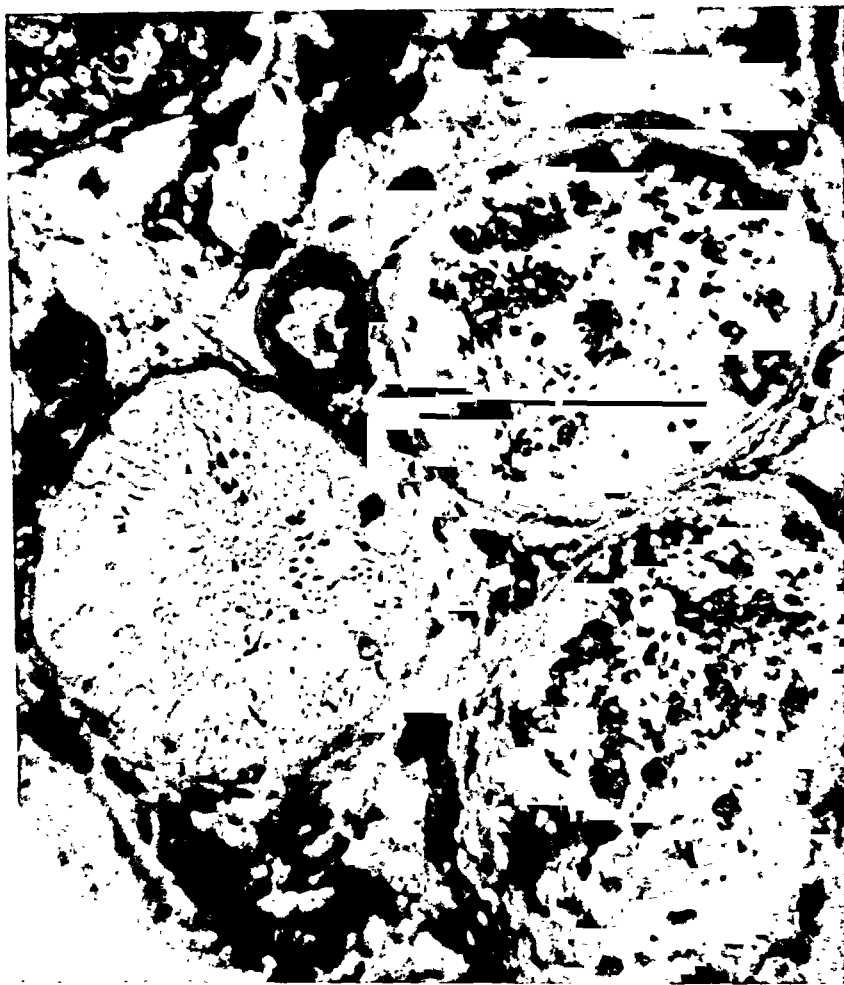


FIG. 2b. Biopsy specimen of testis from patient K. H. during antihormone formation: lower left area of Figure 1b, $\times 450$. Note that spermatogenesis has ceased at the primary spermatocyte stage, and that dark-staining sperm are retained in the tubules. Small cells with pyknotic nuclei are present in the two right-hand tubules. Leydig cells are not seen.

sperm was encountered in R.C.'s specimens, data on his sperm counts are not presented.) Although abnormalities in sperm morphology and motility were observed in some of the patients, no significant alterations occurred during or after therapy.

Great variations in the number of sperm were observed before, during

morphology of the sperm encountered in some of the patients. The lack of decided improvement, despite the fact that the 7 subjects seemed suitable candidates for FSH therapy, may be due to 1) insufficient amounts of FSH administered, 2) the formation of antihormones which interfered so early that the time allowed for stimulating spermatogenesis may have been inadequate, or 3) the defects in spermatogenesis may not have been amenable to correction with gonadotropins.

The slight rise in average numbers of sperm occurring in 5 patients and the sharp rise in 1 lend encouragement to the possibility that FSH therapy

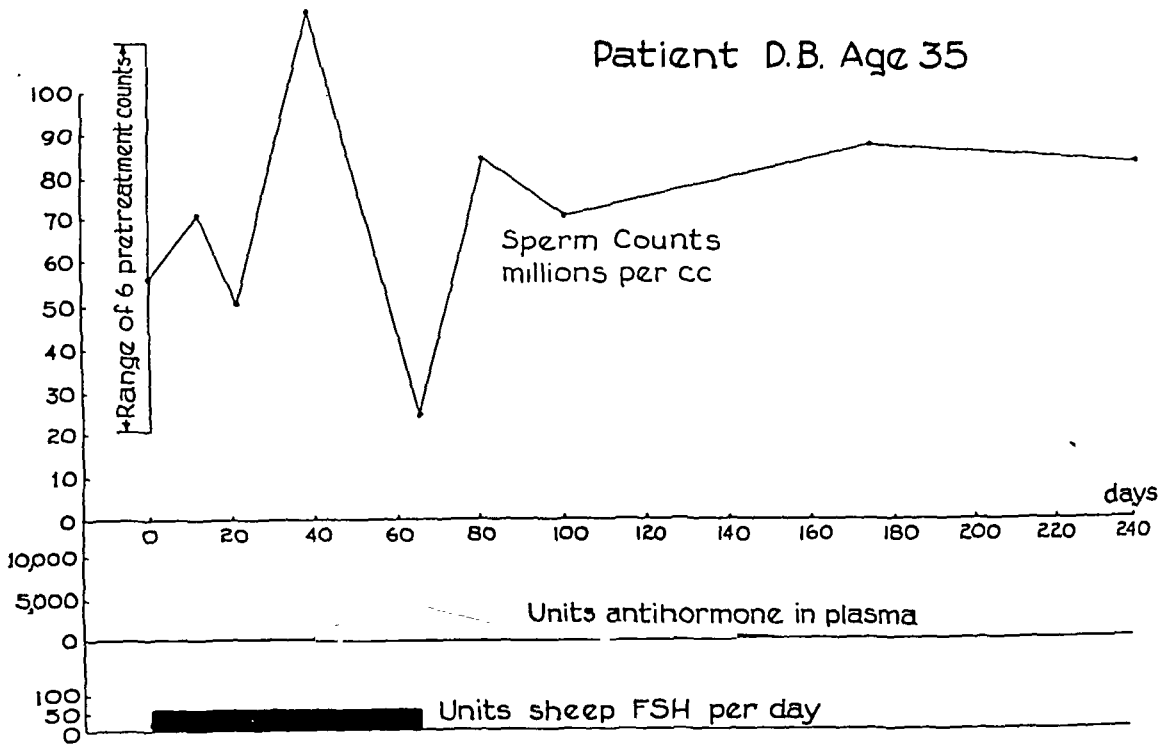


FIG. 3

is potentially capable of stimulating spermatogenesis. The fact that an early plateau or early decline occurred could be correlated with the presence of antihormones in most instances. Thus, one limiting factor in adequate therapy appears to be the formation of antihormones. To circumvent early antihormone formation, perhaps other sources of gonadotropins or more highly purified forms of FSH, less apt to elicit antihormone formation (5), could be applied.

It would appear that antihormone formation did not cause permanent suppression of spermatogenesis, since output of sperm increased to pre-treatment levels or above after the antihormones disappeared from the circulation.

The effects of antihormone formation on the microscopic appearance of

TABLE 1. URINARY GONADOTROPIN ASSAYS BEFORE, DURING AND AFTER FSH THERAPY AND ANTIHORMONE FORMATION

Patient	Anti-hormone formation	Relation to FSH administration	Time elapsing between initiation of therapy and collection of urine (days)	Assay rats*		
				Uterine weight mg.	Ovarian weight mg.	Number of rats
D.B.	0	Before	0	47	11	7
	+	After	65-68	56	10	4
	0	After	185-188	110	12	4
H.B.	0	Before	0	108	21	7
	+	During	66-69	58	9	4
	+	During	101-104	130	12	4
	+	After	141-146	115	22	3
	0	After	241-244	141	26	4
R.C.	0	Before	0	63	28	8
	0	During	45-49	90	13	4
	+	During	81-84	78	14	4
	+	After	87-92	59	13	3
	+	After	138-147	86	12	3
	0	After	200-203	84	44	3
L.D.	0	Before	0	123	23	8
	+	During	63-66	76	10	4
	+	After	83-88	173	22	3
	+	After	179-184	107	69	3
C.G.	0	Before	0	118	38	4
	+	After	64-67	135	16	2
	+	After	96-99	101	51	2
	+	After	167-172	126	45	4
	+	After	186-191	109	24	3
	+	After	249-253	100	64	3
	+	After	282-288	89	77	3
K.H.	0	Before	0	120	12	4
	+	During	64-66	36	8	3
	+	After	69-72	41	8	4
	+	After	73-76	113	12	2
	+	After	185-190	125	23	3
	+	After	218-221	120	18	4
G.M.	0	Before	0	72	18	7
	0	During	26-29	92	19	4
	+	During	54-56	133	23	4
	+	After	71-76	116	25	3
	+	After	162-168	125	17	4
Uninjected control rats				36	13	63

* Each rat received the ultrafilter concentrate of a 12-hour urine specimen.

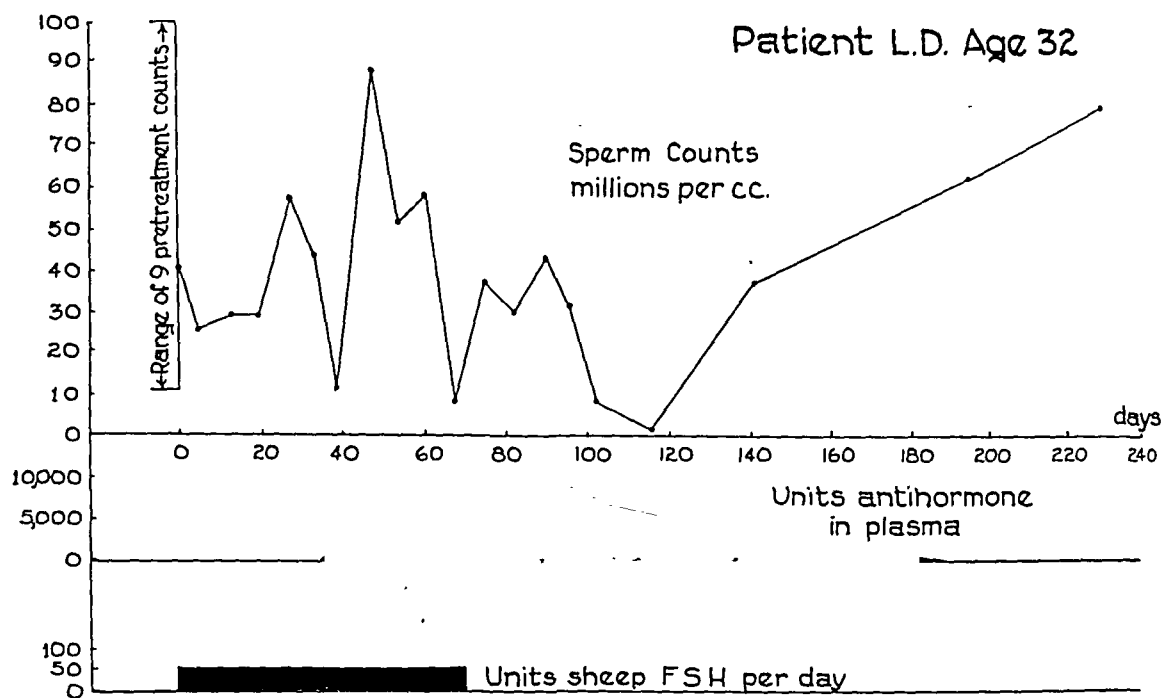


FIG. 5

mis to store sperm may explain this apparent discrepancy. Hammond and Asdell (8) isolated the epididymis from the testis in rabbits. They found that the rabbits remained fertile for as long as forty days after the operation (during which interval they were mated 6 times) and that motile sperm remained in the epididymis for as long as sixty days after operation.

The regression of the interstitial cells of Leydig suggests that either the

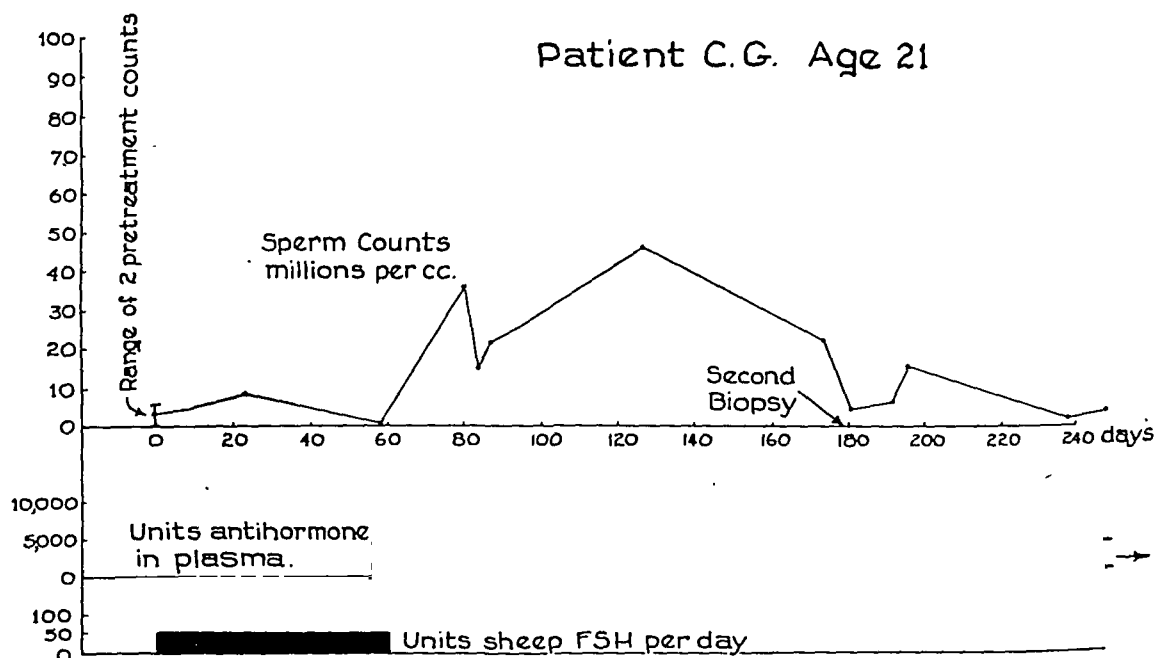


FIG. 6

the testis were studied in two instances in which biopsies were repeated after therapy when antihormones were present. In patient C.G., the tissue was removed for biopsy on day 179, and in patient K. H., on day 67 after initiating therapy. In both patients, sperm counts were decreasing at the time the biopsy specimen was obtained.

Similar changes occurred in both patients and consisted of a reduction in size of the seminiferous tubules, reduction in the number of mature cells of the germinal series leaving a preponderance of spermatogonia and primary spermatocytes, sloughing of a large number of immature cells into

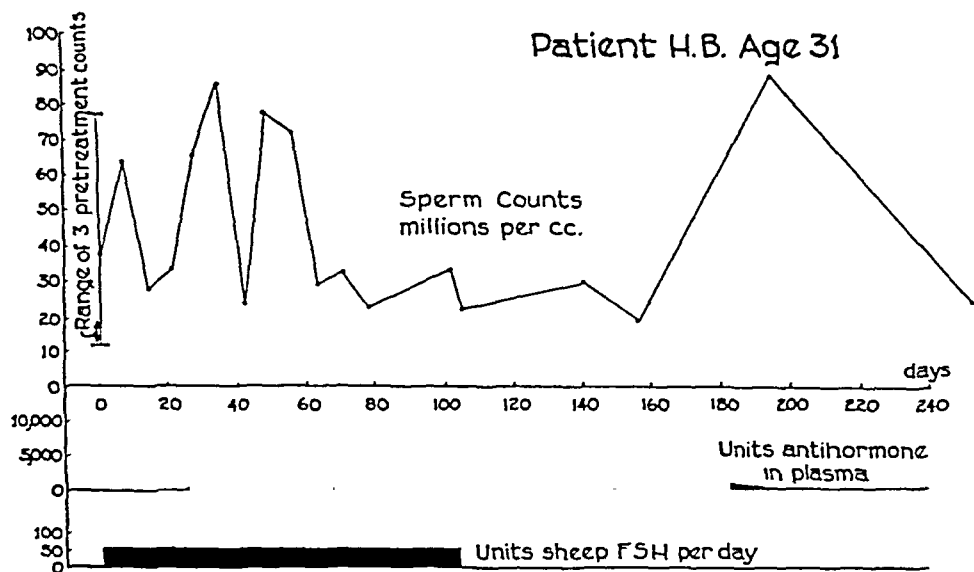


FIG. 4

the lumen of the tubules, an apparent retention of mature spermatozoa (which stained deeply and seemingly were old forms), and disappearance of recognizable interstitial cells of Leydig (Figures 1 b and 2 b, patient K. H.). The general appearance was similar to that encountered in pituitary failure in the human adult (6). The appearance was not unlike that seen in adult hypophysectomized animals (7).

The obvious explanation for the marked regression of the seminiferous tubules is that the antihormones prevented stimulation of the tubules by either the exogenous sheep FSH or the patients' endogenous FSH. Thus, as concerns the seminiferous tubules, the patient had been essentially hypophysectomized.

As judged by the biopsy of the testis, spermatogenesis appeared to have ceased, yet the seminal fluid contained sperm. The capacity of the epididy-

ently androgen production was not markedly interfered with for any great length of time, since none of the patients experienced androgen withdrawal symptoms.

Urinary gonadotropins were tested during FSH therapy to determine whether appreciable amounts of hormone were being excreted. Since gonadotropic titers were lower during treatment than before treatment, it seems reasonable to conclude that no appreciable amounts of the injected sheep FSH were excreted in the urine.

The decrease during FSH therapy cannot be accounted for by anti-hormone suppression. This was concluded from the fact that in one case the decrease occurred prior to antihormone formation, and from the fact that gonadotropins increased to pretreatment levels during the presence of maximal antihormone titers soon after FSH therapy was stopped.

The rise in the level of gonadotropins which was encountered in 4 of the patients during the time of declining antihormone titers or soon after antihormones disappeared, can be explained by the findings of Meyer, Kupperman and Finerty (9). They noted that upon injecting antihormones into rats, a rise in the pituitary content of gonadotropins occurred. Upon stopping the injections of antihormones, evidence of increased secretion of gonadotropin was obtained, following which the gonadotropin content of the pituitary decreased. They concluded that the increased pituitary secretion of gonadotropins was due to the decrease in gonadal function caused by administering antihormones.

SUMMARY AND CONCLUSIONS

Criteria have been set forth for the selection of candidates for treatment of male infertility with a purified preparation of sheep anterior pituitary glands containing mainly follicle-stimulating hormone (sheep FSH). These criteria require that:

1. Biopsies of the testis should reveal a suitable substrate for the action of the FSH, *i.e.*, testicular defects should appear to be potentially reversible.

2. Gonadotropin production should not be elevated. In such instances, addition of exogenous FSH would be superfluous.

Seven sterile men were judged to have fulfilled these prerequisites. They therefore were given daily injections of 50 units of sheep FSH for two to three months.

The average number of sperm increased somewhat initially, and then remained stationary or decreased. The lack of progressive rise seemed to coincide with the presence of circulating antihormones; as the antihormones disappeared, sperm production increased in those instances in which it had formerly declined.

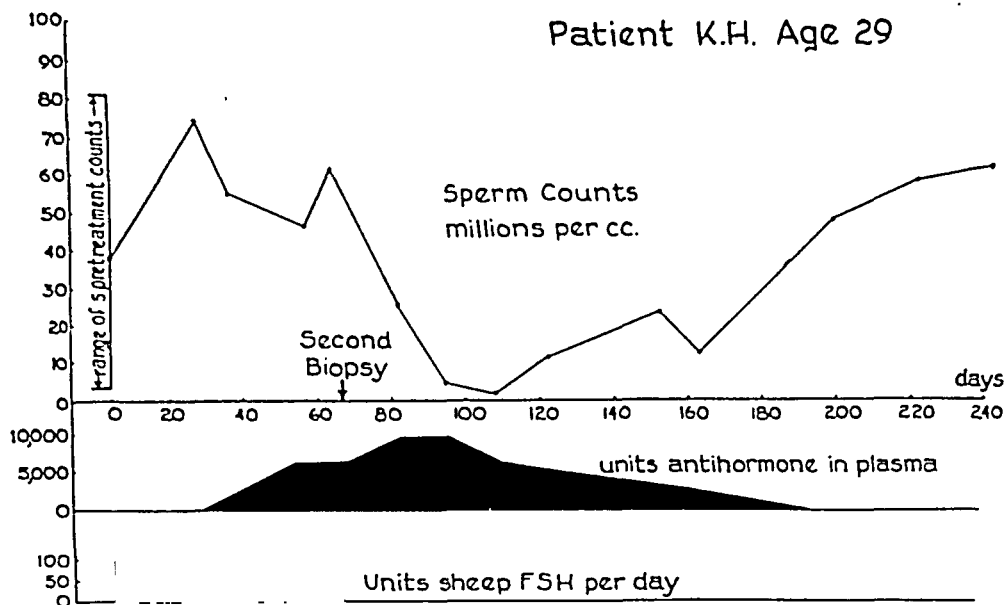


FIG. 7

sheep FSH contained enough interstitial-cell stimulating hormone (ICSH) to cause anti-ICSH formation, thus preventing stimulation of the Leydig cells by endogenous ICSH; or the antihormones to FSH were sufficiently non-hormone-specific to prevent endogenous ICSH stimulation. Appar-

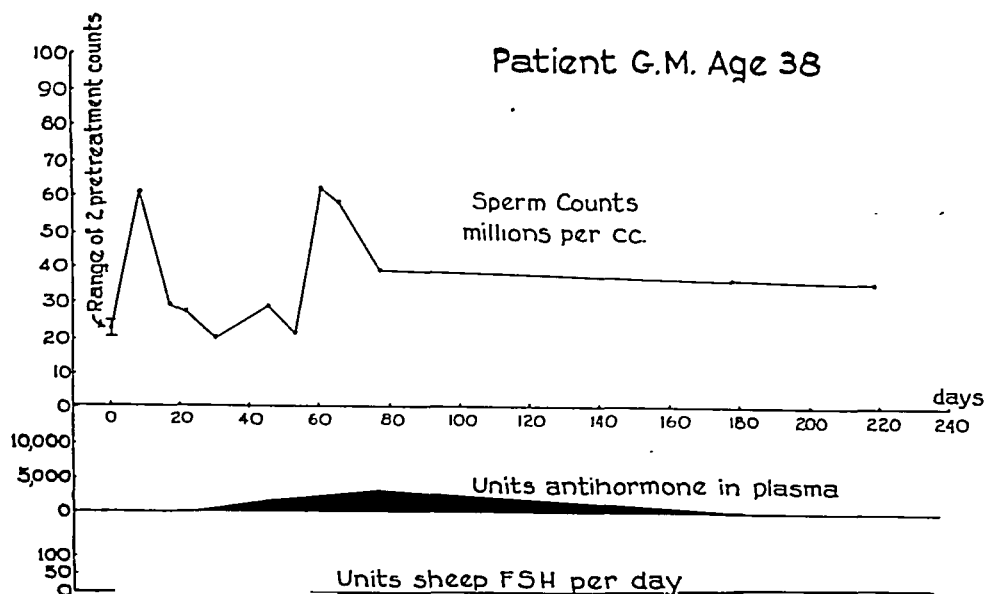


FIG. 8

THE RELATIVE INDEPENDENCE OF SODIUM AND CHLORIDE EXCRETION*

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IN THE early days of electrolyte studies, the concept of "salt" (*i.e.*, sodium chloride) metabolism was widely used. This is exemplified by Zondek's "salt-and-water obesity," a study which was based entirely on chloride determinations (1). Since that time it has been noted that sodium and chloride levels do not always follow a parallel course. In shock and in alkalosis, profound changes in blood chlorides occur with relatively little alteration of the sodium level. In cases of adrenal tumor, low plasma chlorides have been associated with high (2) or normal (3) plasma sodium values. Differences between the urinary excretion of sodium and chloride have been observed very recently in cardiac edema and in hepatic cirrhosis (4). Nevertheless, the habit of judging sodium metabolism by chloride values, and the expression of chloride concentrations as "milligram per cent of sodium chloride" or chloride output "as sodium chloride" persists. Extensive work on the relation of steroid hormones to electrolyte balance, as well as renewed interest in sodium metabolism as related to heart failure and hypertension, have emphasized the need for separate evaluation of sodium and chloride balance. With the availability of a simple method for the determination of sodium in biologic fluids, simultaneous balance studies of sodium and chloride were carried out to determine how frequently these substances are treated by the body in parallel fashion.

MATERIALS AND METHODS

Fifty consecutive patients referred for endocrine investigation of various problems were studied. This potentially abnormal material was selected purposely because of the greater likelihood and importance of finding irregularities of electrolyte metabolism. The patients were maintained on identical diet and fluid intakes for two consecutive days during which 24-hour urine samples were collected. The first day was taken as a control, and on the second day 10 Gm. (0.188 mol) of sodium chloride in gelatin capsules and 250 cc. of water were given between 9 and 10 a.m. Total sodium excretion was determined by the method of Goldzieher and Stone

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Biopsy specimens of the testis from 2 patients, taken at a time when anti-hormones were present and when sperm counts were decreasing, revealed a microscopic appearance not unlike that encountered following hypophysectomy in adult animals.

Urinary gonadotropin excretion was determined before, during and after therapy. The injected sheep FSH was not excreted in the urine in appreciable quantities. Antihormones did not suppress endogenous gonadotropin excretion.

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and chloride retention correspond within ± 5 per cent. Moreover, it appears that patients with relatively little sodium retention may nevertheless retain a great deal of chloride: of 9 patients with 0 to 30 per cent sodium retention, 4 showed a chloride retention of 60 per cent or more. On the other hand, chloride retention seldom keeps pace with an intense sodium retention: of 22 patients with a sodium retention in excess of 60 per cent, only 1 showed a relatively greater chloride retention and 15 showed considerably less chloride retention than expected.

An attempt was also made to correlate the retention of the test dose of water with retention of sodium, chloride or both. However, there were so many uncontrolled variables affecting the urine volume (such as atmospheric temperature and skin evaporation) that the variations were too great to yield statistically significant results in a group of this size.

DISCUSSION

It is a well established observation that blood levels of sodium and chloride often run a parallel course. This observation, and our preoccupation with the study of sodium, potassium and chloride often lead us to ignore the obvious fact that these electrolytes form only a part of, and are dependent upon changes of the total acid-base balance. This fact accounts for the observation of chloride retention without sodium retention and vice versa. It also serves to point out that there is actually no such thing as "sodium chloride" in the electrolyte system and that this term is based on a misconception which antedates our present concepts of acid-base balance.

Tests based on electrolyte balance are commonly used in the diagnosis of pituitary disease, adrenal cortical disease and other conditions. All of these tests rely on chloride changes as the sole criterion or as part of the final equation (*e.g.* the Robinson-Power-Kepler test). One is led to wonder whether or not the substitution of sodium for chloride determinations might not augment their sensitivity.

SUMMARY

The simultaneous urinary excretion of administered sodium and chloride was studied in 50 patients who were referred for endocrine study. Excretion of equivalent amounts of sodium and chloride was found in only 26 per cent. A significant number showed sodium retention without chloride retention and vice versa. It is concluded that these two electrolytes frequently behave in a relatively independent fashion, and that the behavior of one cannot safely be deduced or predicted from investigation of the other element.

(6) and total chloride by the method of Schales and Schales (7, 8). From these data, the percentage excretion of the test sodium and chloride could be calculated.

RESULTS

The data obtained are shown as a scatter diagram in Figure 1, the retention of the test sodium being plotted as the abscissa, and the retention of the test chloride as the ordinate. It is usually assumed that with equal

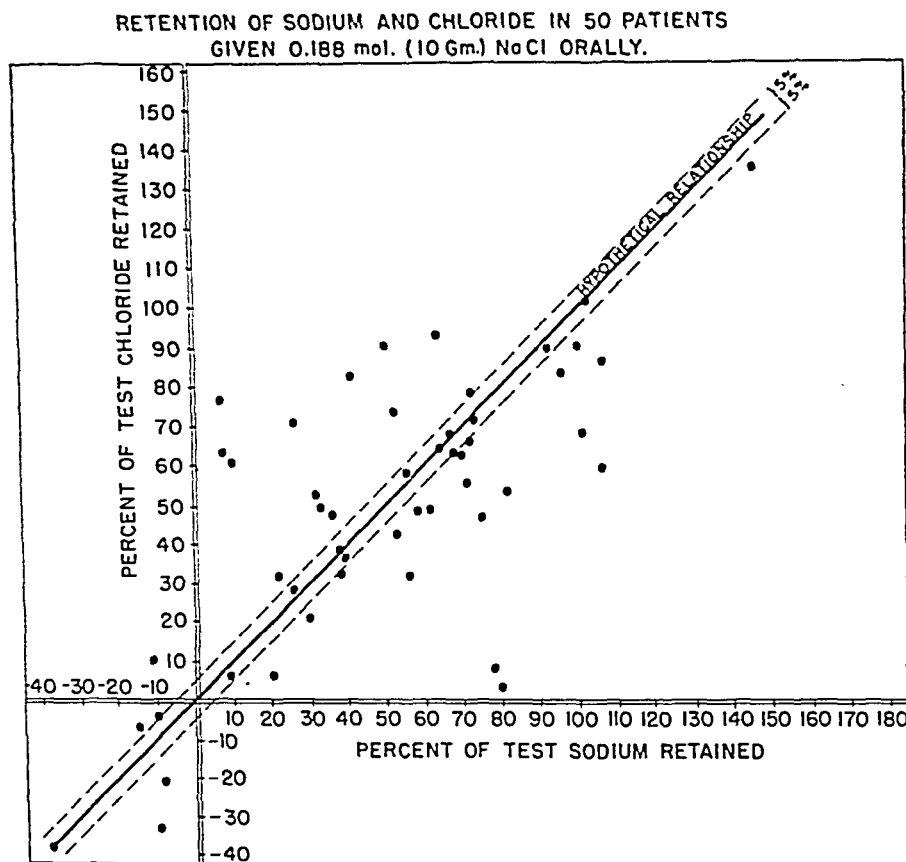


FIG. 1

amounts of sodium and chloride ingested, the percentage retention or excretion of one should be essentially identical with the percentage retention or excretion of the other. This hypothetical situation would yield a series of values which form a straight-line relationship, as illustrated. It is found, however, that very few of the actual observations conform to this assumption. In 4 cases (8 per cent), equal fractions of sodium and chloride were excreted. In only 9 other patients (18 per cent) did the amounts of sodium

THE EFFECT OF TESTOSTERONE PROPIONATE IN A CASE OF PITUITARY TUMOR OBSERVED FOR NINE YEARS*

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IT HAS been demonstrated that pituitary tumors, particularly chromophobe adenomas, can be produced experimentally in animals by prolonged administration of estrogens. This was shown by Cramer and Horning (1), Zondek (2, 3), and Wolfe and Wright (4). Experimental evidence has also been presented by Wolfe and Hamilton (5, 6) that androgens have an inhibitory effect on estrogens in pituitary physiology. However, the value of androgens in ameliorating the symptoms of intracranial pressure caused by a chromophobe adenoma in man has not yet been demonstrated.

The case described here is of interest because of the possible inhibitory effect of testosterone propionate on a pituitary tumor, clinically of the chromophobe type.

CASE REPORT

The patient, W. J., (Fig. 1) a eunuchoid male, 24 years of age, was admitted to the Endocrine Clinic of the Pasadena Dispensary on March 24, 1939. He stated that he had had normal development and hair growth at 15 years of age, having been examined by a school physician at that time. Soon after that he noticed testicular atrophy with gradual disappearance of pubic and axillary hair.

In 1939 he began to experience frontal headaches, which seemed to begin behind the eyes and were worse on the left side. These headaches were of intense, boring, pressing nature. They would last about half an hour and recur three to four times a day. The headaches had been getting progressively worse up to the time of admission. These symptoms were accompanied by a feeling of extreme muscular weakness. The family history was normal except for a thyroidectomy performed on the patient's mother in 1925.

Physical examination

The patient was an apathetic, anemic-looking eunuchoid male, 24 years of age, whose skin had a peculiar, startling pallor. He had a high-pitched boyish voice, and the typical eunuchoid skeletal development and measurements. His height was $70\frac{1}{4}$ inches, his span was $70\frac{1}{4}$ inches, the pubis-to-vertex measurement was $30\frac{3}{4}$ inches, and the pubis-to-floor measurement was 39 inches. His weight was $121\frac{1}{2}$ pounds and blood pressure, 80/60.

There was atrophy of the left optic disc and bilateral temporal hemianopia, more pronounced on the left side. Visual fields were outlined by Dr. Henrietta Johnson on March 27, 1939; they showed marked contraction of the left fields and moderate contraction of the right.

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* A preliminary report on this case was presented before the Los Angeles Society of Neurology and Psychiatry, Feb. 19, 1941.

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R.B.C. 4,040,000; W.B.C. 12,250; neutrophils 40 per cent; monocytes, 5; lymphocytes, 53; and eosinophiles 2 per cent. The basal metabolic rate was minus 9 per cent.

Roentgenograms (Fig. 2) taken on May 9, 1939 showed that most of the epiphyses

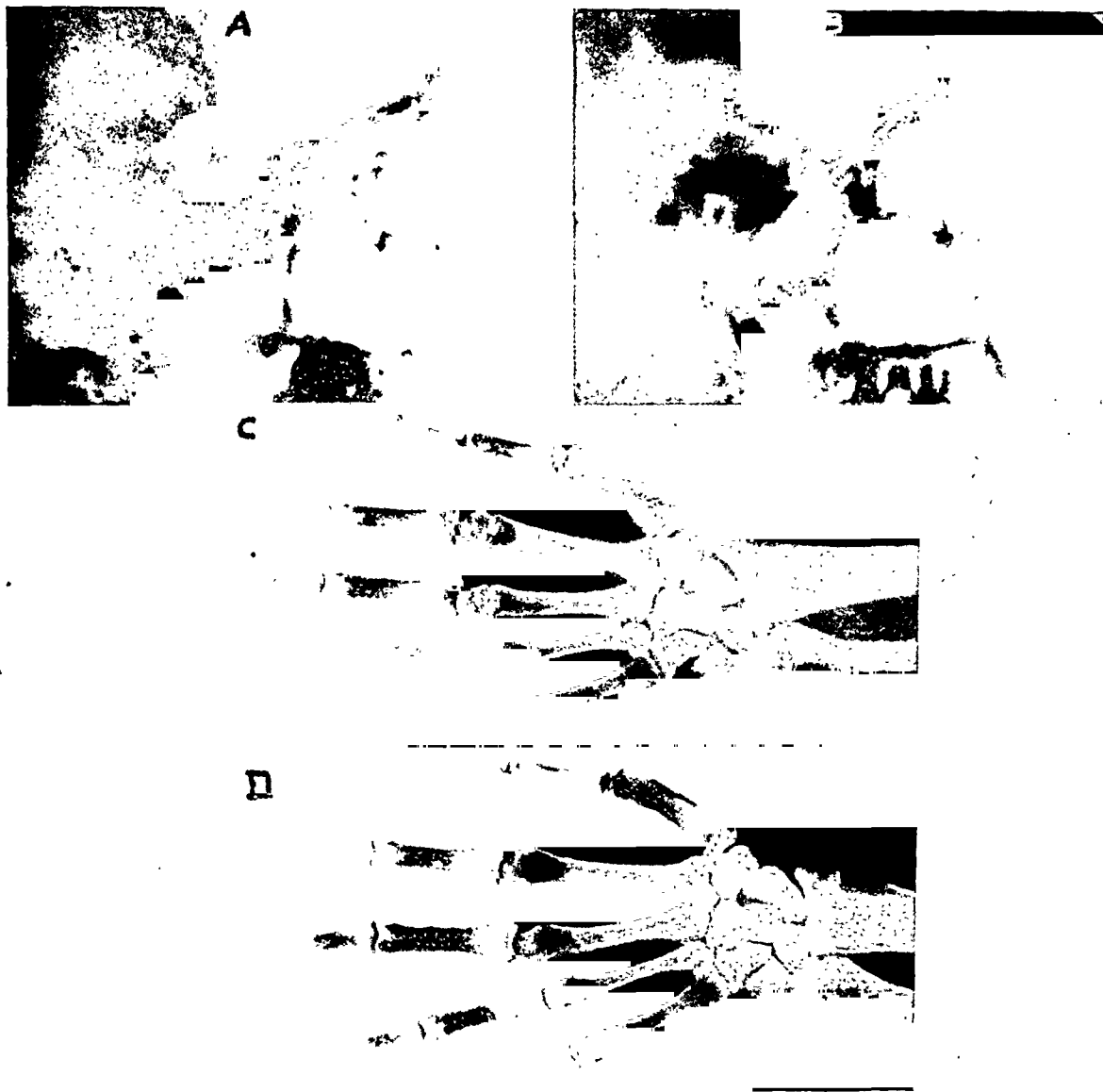


FIG. 2. A. Sella turcica before treatment, showing marked enlargement and erosion of clinoids. B. Sella turcica nine years later. C. Hand, before treatment, showing nonunion of phalangeal and radial epiphyses. D. Complete union of epiphyses nine years after beginning of treatment.

were open, with bone age retardation of 7 years. The sella turcica revealed extensive destruction with displacement of the pineal gland posteriorly. A diagnosis of pituitary tumor was made.

Treatment

At first the patient was treated with 10 mg. of testosterone propionate¹ intramuscu-

¹ I wish to thank Dr. William H. Stoner of the Schering Corporation for supplying the testosterone propionate (Oreton) used in this case.

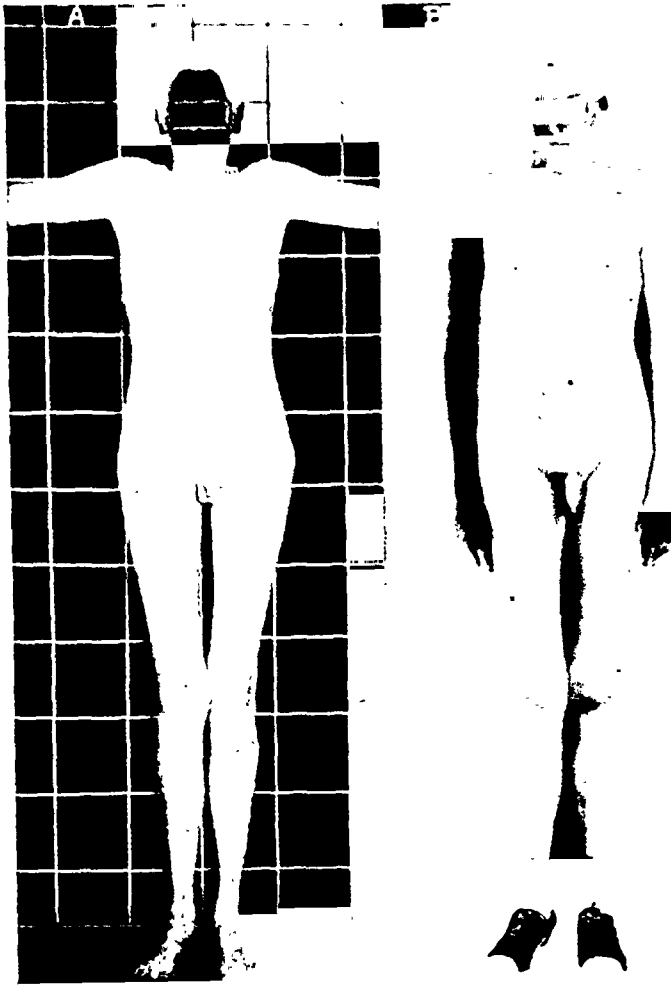


FIG. 1. A. Patient W. J., age 24, before treatment. B. After fifteen months of treatment with testosterone propionate.

The development of the penis and testes was retarded, with absence of pubic and axillary hair. The testes were both down in the scrotum and were about $1\frac{1}{4}$ cm. in diameter.

Heart, lungs and abdomen showed no abnormalities.

Laboratory findings

On April 4, 1939, the concentration of serum calcium was 10.2 mg. per cent; serum phosphorus, 3.8 mg. per cent; and plasma cholesterol, 248 mg. per cent. A sugar tolerance test showed 76 mg. per cent for the fasting blood, and 120 mg., 150 mg. and 118 mg. per cent respectively for the $\frac{1}{2}$ hour, 1 hour and 2 hour blood samples. All urine specimens were free of sugar.

A blood count gave the following results: Hgb. 11.3 Gm. (66.9 per cent Newcomer);

From May 24, 1940 to October 6, 1941 the patient received intramuscularly 2300 mg. of testosterone propionate in oil.

Neurologic examination by Dr. C. Hunter Shelden on December 19, 1940 showed atrophy of the left optic disc. Central field studies using a screen revealed typical evidence of a developing chiasmal lesion. A minimal upper outer quadrant depression was found in the right eye, whereas the field for the left eye showed a complete temporal anopsia as with a central scotoma. The test object used was a white 3 mm. bead. In this examination the field of the left eye was 3 degrees to the nasal side of the fixation. A second field study was made by Dr. Shelden on February 5, 1941. At this time Dr. Shelden reported disappearance of the central scotoma noted previously. The vision in the left eye was found improved 50 per cent.

Dr. Cyril B. Courville also examined the patient on June 13, 1941 and confirmed Dr. Shelden's findings. Both neurologists agreed that surgery should be postponed because of the improvement in the visual fields and disappearance of headaches.

Seen again on July 31, 1942, the patient was energetic, free of headaches and working full time.

At the present time (July 1948) he is working regularly as a public accountant.

SUMMARY AND CONCLUSIONS

A eunuchoid male, 24 years of age, with a pituitary tumor clinically of chromophobe type received a total of 3400 mg. of testosterone propionate intramuscularly between April 14, 1939 and October 6, 1941. The following results were noted during testosterone therapy before x-ray therapy was begun: widening of visual fields with disappearance of headaches, closure of the epiphyses, moderate increase of sexual development, gain in weight and strength and great improvement in mental outlook.

Although the results in this case are promising, the problem of whether or not androgens have an inhibitory effect on chromophobe adenomata in man needs further investigation.

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larly three times a week. From April 14, 1939 to May 24, 1940 the patient received 1090 mg. of testosterone propionate intramuscularly, with resulting increased strength and energy, complete cessation of headaches, moderate growth of pubic and axillary hair with increase in the size of testes and penis. During this interval the patient had obtained a position as a filing clerk, where he had to use his eyes constantly. Visual fields outlined by Dr. Johnson on February 26, 1940 showed considerable improvement in both eyes.

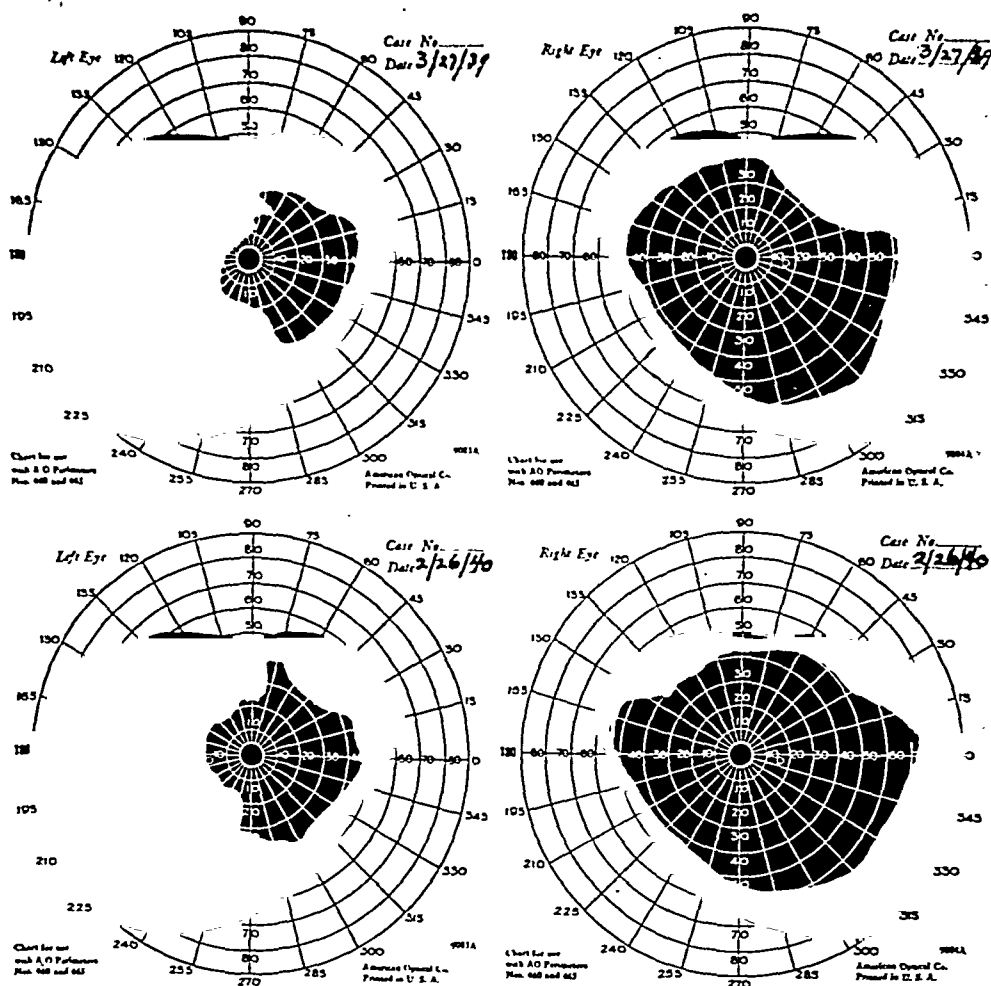


FIG. 3. Above: Visual fields before treatment. Below: After eleven months of treatment with testosterone propionate.

In the hope of further improvement in the visual fields the patient was given seven x-ray treatments to the pituitary of 150 roentgens each from May 24, 1940 to June 13, 1940 by Dr. John Chapman at the Huntington Memorial Hospital of Pasadena. The following factors were used: 220 K.V., 15 M.A. 2 mm. cu., 1 mm. al filter, 50 cm. distance, 7 circle port, 4 min. 30 sec. for 100 r., 7 min. for 150 r.

Visual fields rechecked by Dr. Johnson on June 10, 1940 revealed further improvement of both fields.

pressure 108/78. During this interval the patient continued to feel perfectly well, and her only complaint was failure to lose weight despite strict adherence to a 1000 calorie diet. Her weight at this time was 229 pounds, one pound more than she had weighed on admission to the hospital.

On July 18 an 800 calorie diet was prescribed which the patient faithfully followed with gradual loss of 25 pounds in the ensuing two months. On July 28 the patient experienced transient dizziness when she suddenly rose from a supine position but this experience was not repeated and in general she felt well and happy. The following day the blood serum contained 4.5 gamma per cent precipitable iodine and 6.7 gamma per cent total iodine (total iodine was determined in a separate laboratory). The menstrual period due August 20 was delayed approximately twenty days, an event so unusual that the patient was concerned about possible pregnancy but a *Xenopus laevis* pregnancy test on September 5 gave negative results. When menstruation finally began on September 9 the period was normal in all respects. The next period appeared October 5 and again was "normal" in duration and quantity. Catamenia have been regular since that time. Pulse rates and laboratory findings are recorded in Table 1.

TABLE 1

Date 1947	Pulse	Blood pressure mm. Hg	Weight lbs.	B.M.R. per cent	Serum iodine gamma/100 cc.	
July 10	110	130/85	228	—	—	—
July 11	85	112/72	228	+ 4	{ Precipitable	40.9
					{ Filtrate	130.9
July 15	86	116/78	228	- 3	—	—
July 18	96	108/76	229	—	—	—
July 22	86	—	227	—	—	—
July 29	88	—	220	-14	{ Precipitable	4.5
					{ Total	6.7*
Aug. 5	76	112/74	222	—	—	—
Aug. 12	80	124/84	223	—	—	—
Aug. 16	80	126/84	219	—	—	—
Aug. 22	80	118/86	216	-10	—	—
Sept. 22	76	114/78	205	-15	—	—
Oct. 31	88	112/86	196	- 9	—	—

* Total iodine determined in a separate laboratory.

DISCUSSION

The patient ingested 63 grains of "Proloid" containing approximately 8.4 milligrams of organic iodine. Since equi-iodal amounts of thyroid globulin and of racemic thyroxine have been found to be equal in calorigenic potency when tested in myxedematous subjects (7), a dose of 13.3 mg. of thyroxine intravenously would probably be necessary to equal the dose of "Proloid" assuming, of course, that the patient was actually

ABSORPTION AND CLINICAL EFFECT OF A LARGE SINGLE DOSE OF THYROID GLOBULIN

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PREVIOUS papers have described a clinical condition similar to hyperthyroidism resulting from continuous ingestion of excessive amounts of desiccated thyroid (1, 2, 3, 4, 5, 6).

In the case to be reported there were no toxic effects, although a serum precipitable iodine of 40.9 gamma per cent established the fact that a great part of a single huge dose of thyroid globulin had been absorbed.

REPORT OF CASE

The patient, M. W., a 31-year-old married white woman, consulted her physician in the latter part of June 1947 regarding her obesity. For two years following her first parturition in 1934, when she was 18 years old, she had weighed 117 pounds. After a right salpingo-oophorectomy and appendectomy at the age of 20, and following a second pregnancy in 1938, her weight was 160 pounds. This increased after her third pregnancy in 1945 to 248 pounds.

The patient's physician prescribed a low caloric diet and 3 grains of thyroid daily. During the first nine days of July 1947 the patient took a total of 27, one-grain, "Proloid" (Maltine) tablets. On the morning of July 10, 1947 the patient took all the tablets remaining in the box, presumably 63 tablets, in a single dose. Promptly repenting, she called a physician, who hospitalized her for observation of the possible evolution of thyrotoxicosis. Twenty-four hours after the ingestion of the drug the basal metabolic rate was plus 4 per cent, at which time the serum contained 40.9 gamma of precipitable iodine and 130.9 gamma filtrate iodine per 100 cc. The serum cholesterol was 116 mg. per cent. Red blood cells numbered 5,100,000 with 13.5 Gm. hemoglobin. White cells were 4,700 with 39 per cent segmented polymorphs, 8 per cent stem cells, 51 per cent lymphocytes and 2 per cent monocytes. The Kahn test was negative. Analysis of a concentrated specimen of urine (sp. gr. 1.030) revealed no abnormalities. The patient was apprehensive on admission when an electrocardiogram was taken, which probably accounts for the tachycardia (110 beats per minute) noted in the tracing. While lying quietly in her hospital bed the pulse rate remained at or about 80 per minute with a maximum of 90. Blood pressure was 130/85 on admission but shortly dropped to levels between 110 and 118 systolic and 68 to 78 diastolic, where it remained during the three days of hospital residence.

Further hospitalization was impossible and likewise bed rest at home was unfeasible so that precise observations of possible changes in circulatory dynamics could not be continued. The patient was able to make frequent visits to the physician's office and on her first visit, two days after discharge from the hospital and the fifth day after the overdose of thyroid, the pulse was 90 per minute, blood pressure 112/76 and the basal metabolic rate minus 3 per cent. Three days later the pulse was 96 per minute, and blood

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* Present address: 4 East Congress, Tucson, Arizona.

ported, in which patients have taken large amounts of thyroid either because of "despondency" or a desire to commit suicide. Goldfinger reported the death of a woman who had taken as much as 100 grains daily (5). Thompson (4) described at length the intake of 80 to 120 grains daily by a physician who subsequent to this self medication developed symptoms of hyperthyroidism and of "psychosis." These cases are cited to emphasize that, although the patient described in this report exhibited no serious symptoms after one massive dose of thyroglobulin, the dangers of self medication with desiccated thyroid can not be ignored. This is only one reason why the present day practice of prescribing desiccated thyroid should be restricted for use by suitable patients.

CONCLUSIONS

A case is reported of a patient who, after ingestion of 63 one-grain tablets of "Proloid" (Maltine), did not develop thyrotoxic symptoms, in spite of a protein-bound iodine of 40.9 gamma per 100 cc. of serum.

From the literature are cited two cases in which continuous ingestion of desiccated thyroid in massive amounts produced thyrotoxic symptoms.

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myxedematous and that absorption from the gut was complete. Complete absorption cannot be proved from the data in this case but the frankly colossal values for total iodine and precipitable iodine in the serum twenty-four hours after ingestion indicate that at least a large part of the thyroid globulin was actually absorbed. Nineteen days later the value for precipitable iodine as well as for total serum iodine had fallen to normal levels.

The fate of the excessive circulating hormone during this interval is not known but from "decay" curves and excretion studies (8, 9) it may be assumed that precipitable iodine concentration diminished rapidly with probable progressive conversion of the protein-bound fraction to the filtrate fraction. It is regrettable that more frequent iodine studies could not have been made.

Of chief interest is the total lack of symptoms and signs of thyrotoxicosis at any time following the absorption of a dose of active thyroid globulin roughly equivalent to a 13.3 mg. dose of racemic thyroxine. Thompson and co-workers (9) gave a single intravenous dose of 10 mg. of thyroxine to a female patient with fully developed myxedema and produced a rise in the basal metabolic rate from minus 45 per cent to minus 6 per cent in seven days with a gradual return to a level of minus 30 per cent over a period of one month. It is reasonable to assume that had the present patient been suffering from true thyroid deficiency of any degree there would have been a detectable response to the huge dose of "Proloid" ingested and absorbed. It is well established that euthyroid individuals have a tolerance for thyroid medication at dosage levels which provoke toxic symptoms in hypothyroid individuals (9, 10, 11). The difference in response to thyroid therapy frequently is the only critical method of differentiating hypometabolism due to thyroid deficiency and hypometabolism due to other causes.

In the present case there is clear evidence of high tolerance and it seems likely that it may be ascribed at least in part to the presence of a normally functioning thyroid gland. Even in a normal euthyroid individual a response to at least the thyroxine fraction of the ingested dose (2.99 mg. of thyroxine in 63 grains "Proloid")¹ could be expected (12). The nature of the conversion of the active hormone to an iodine-containing organic compound present in the blood but incapable of increasing oxidative metabolism of the tissues would be entirely speculative from the data in this case. Recent studies (13) with labelled iodo-casein would suggest that the liver plays an important role in the intermediary metabolism of thyroid hormone.

Two previous rather similar cases, but without determinations of serum precipitable iodine as a measure of circulating hormone have been re-

¹ Based on a letter from Walter Hoskins, Ph.D., Director of Research Laboratories, The Maltine Company, Morris Plains, New Jersey.

Letters to the Editor

TO THE EDITOR:

THE BUCCAL ADMINISTRATION OF ESTRADIOL

HAVING previously reported that the buccal administration of estradiol in a propylene glycol-alcohol solution affords effective absorption and utilization in a manner comparable with the same quantity of hormone by intramuscular injection (1), the present report deals with the administration of estradiol in solid polyethylene glycol tablets¹ placed within the mouth and absorbed through the buccal mucosa.

Method

The tablets are composed of a polyethylene glycol wax, in which the steroid hormone is soluble, and which is miscible with the oral fluids. They contain 0.1 or 0.25 mg. of pure alpha-estradiol, the primary estrogen of the natural steroid hormone series. A tablet is placed in the mouth in the space between the cheek and the gum of the lower molar teeth, where it gradually dissolves. Insertion of the tablet just before going to sleep at night provides more uniform absorption than when used during the daytime.

Vaginal smears were appraised in all patients at weekly intervals, according to our method previously described, *viz.*, the vaginal material is collected from the lateral wall of the vagina with a dull, curved spatula; it is spread thinly on a clean glass slide and dried with compressed air. The smear is then stained for 20 seconds with 2 per cent aqueous solution of basic fuchsin. Excess solution is washed off with water, the slide is dried with compressed air and is ready for evaluation. This rapid method enables us to determine advisable dosage without delay and is completely adequate for clinical purposes, as proved by twelve years of experience with over 60,000 slides.

Results

In order to determine the effects of this method of administration in cases of estrogen deficiency, 5000 buccal tablets of estradiol were administered to 103 patients during 280 monthly periods. The patients included those with 1) natural or artificial menopause, 2) pituitary-ovarian deficiency and 3) temporary hypo-estrogenism. No patient was treated with "buccal" estradiol unless the clinical response had first been determined to intramuscular alpha-estradiol benzoate or to oral ethinyl estradiol. All but 4 patients showed adequate symptomatic and vaginal cornification response. These 4 were coincidentally disturbed by emotional stress or infection.

Buccal versus intramuscular alpha-estradiol. Menopausal patients who had been given 1.66 mg. of alpha-estradiol benzoate at weekly intervals soon showed typical responses in the vaginal epithelium and subjective

¹ Materials used in this study were supplied by the Schering Corporation.

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Buccal administration, using the tablet as described, is accordingly less variable in effect. By either method the steroid hormone is absorbed into the systemic rather than into the portal circulation. Physiologically, the buccal route is superior, compared on the basis of the effects of equal doses.

Summary

Alpha-estradiol administered by the buccal route in polyethylene glycol tablets exhibits a high order of clinical efficiency, generally exceeding that of estradiol esters given by injection. This has been demonstrated by the results in 103 patients with estrogen deficiency, in most of whom a daily dose of 0.25 mg. was adequate for control. By this route, as by all other routes used, estradiol is devoid of clinical toxicity.

626 Medico-Dental Building,
Sacramento, California.

GEORGE JOYCE HALL, M.D.
September 30, 1948.

REFERENCE

1. HALL, G. J.: Clinical experiences with the sublingual administration of alpha estradiol, *J. Clin. Endocrinol.* 2: 26-28 (Jan.) 1942.

TO THE EDITOR:

ANTI-HORMONE FORMATION DURING CHORIONIC GONADOTROPIN THERAPY

THE administration of gonadotropic extracts from heterologous sources frequently elicits antihormone formation in man (1) whereas homologous source material does not cause hormone antagonists to develop (2). There are patients whose sera were found to contain substances capable of inhibiting the action of chorionic gonadotropin but in all of these a pituitary extract had also been given (3, 4).

In order to assess possible antihormone formation in response to a highly purified chorionic gonadotropin,¹ serum samples were obtained from patients receiving chorionic gonadotropin for a study of this hormone's effect on the functional life of the corpus luteum (5). One patient received 20,000 i.u. daily from February 12 to March 10 and 10,000 i.u. daily from July 13 to August 3, a total of 760,000 i.u. in two periods of intensive treatment. The patient's serum was tested eleven and thirty-one days after the last injection. The two to three-fold increase in mouse ovarian weight induced by 20 i.u. of chorionic gonadotropin was completely prevented by concomitant administration of the patient's serum. No such antagonistic action, however, could be demonstrated six months after the last injection.

¹ Chorionic gonadotropin (Antuitrin-S) was generously supplied by Dr. D. A. McGinty of Parke-Davis and Company.

improvement of the symptoms of estrogen deficiency. This medication was then stopped and the vaginal condition and symptoms of the menopause recurred. The patients then received the estradiol tablets by the buccal route. A daily tablet of 0.25 mg. of estradiol (1.75 mg. per week) was given during the following month. A more complete vaginal cornification and a more pronounced improvement of the subjective symptoms was noted than with the injection method. When 1 mg. of alpha-estradiol benzoate was administered intramuscularly twice a week for one month and was followed by 0.25 mg. of estradiol in buccal tablets daily for one month, it was again found that the clinical and subjective effects of the latter (1.75 mg. per week) exceeded those of the injected hormone (2.0 mg. per week) in most instances; and in all cases were at least equal.

Buccal alpha-estradiol versus oral ethinyl estradiol: Comparisons were made on the basis of 0.1 mg. of buccal estradiol versus 0.05 mg. of oral ethinyl estradiol. The vaginal epithelium responses after the buccal tablets were always as good as, and in the greater number of cases, better than, those with oral ethinyl estradiol. The subjective improvement was invariably more pronounced with the buccal material.

All of the estrogens mentioned here were used so that a fair comparison could be made. None of them gave as complete relief subjectively and objectively as was experienced with the new tablet preparation.

All patients using the buccal tablets experienced an increased feeling of well being. All subjective symptoms due to estrogen deficiency were controlled, and many patients volunteered the information that they had not felt so well in a long time, even when receiving intramuscular injections regularly. No untoward effect of any kind was reported. In menopausal patients symptoms were well controlled; and in those with other ovarian disorders menstrual function was improved. Nervous symptoms were controlled or improved to the point of complete comfort. Vulvar and anal dermatitis (3 patients) disappeared when the vaginal epithelium attained the condition seen in full estrus. Objectively, there was uniform improvement in the vaginal smear, comparable to the subjective improvement. As judged by the smears, all patients showed adequate estrogenic response and a few showed some degree of hyperestrogenic activity, indicating the need for reduced dosage.

Because of the efficiency of the buccal tablets it became increasingly apparent that estrogen should not be administered for more than three of each four weeks, a plan followed here for many years.

While sublingual administration of estradiol in solution in propylene glycol and alcohol affords clinical activity of the same order as that obtained on injection of its esters, the method requires too frequent daily doses and too much dependence on careful cooperation by the patient.

The 1949 Meeting of the Association for the Study of Internal Secretions

FRIDAY AND SATURDAY, JUNE 3 AND 4

Headquarters: Chalfonte-Haddon Hall, Atlantic City, New Jersey.

Registration: Everyone attending the meetings is requested to register. A fee of \$1.00 will be charged non-members of the Association. Membership cards should be presented when registering.

The Scientific Sessions: The Scientific sessions will be held in the Viking Room of the Haddon Hall Hotel and programs will begin promptly on schedule. Papers presented at all meetings are planned for ten minutes, unless otherwise noted, and owing to the heavy schedule must be kept within this limit. Manuscripts of all papers should be submitted to the presiding officer or Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Association will be held on Friday evening, June 3, at 7:30 o'clock in the Rutland Room of the Haddon Hall, preceded by cocktails at 6:30 o'clock in the West Room. Secure tickets at time of registration.

Council Meetings: There will be a meeting of the Council on Thursday afternoon, June 2, at 2:00 o'clock, and a luncheon meeting on Friday, June 3.

Business Meeting: The Annual Business Meeting of the Association and Election of Officers will be held at 4:30 p.m., June 4, in the Viking Room of the Haddon Hall.

Local Arrangements: Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

PROGRAM

FRIDAY, JUNE 3, 1949

9:15 a.m.—C. N. H. Long, presiding

1. Piperido-methyl-benzodioxane (933-F): Some Pharmacological and Experimental Observations. By Evan Calkins (by invitation), George W. Dana (by invitation), J. C. Seed (by invitation) and John Eager Howard.

2. Nor-Epinephrine in Adrenal Medulla. By Marcel Goldenberg and Mogens Faber (introduced by R. F. Loeb).

Thus, in the absence of any other hormone treatment, a highly purified chorionic gonadotropin excited antihormone formation. It is suggested that the manipulation necessary to gain purity may have altered or denatured the hormone so that it could be antigenic in the large amount administered.

Rutgers University,
New Brunswick, N. J.
and
State University of Iowa,
Iowa City, Iowa.

JAMES H. LEATHEM, PH.D.
JAMES T. BRADBURY, PH.D.
January 19, 1949.

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TO THE EDITOR:

INSULIN THERAPY FOR RELIEF OF PAIN IN OSTEITIS DEFORMANS

SEVERAL years ago I suggested the use of insulin in the treatment of Osteitis deformans (Paget's disease). Since then I have continued to use it in many patients in a daily dose of from ten to fifteen units of protamine zinc insulin. The rationale of this therapy is as follows:

It has been found that the majority of patients with osteitis deformans have a lowered sugar tolerance. About one-third of 41 patients with osteitis deformans gave a family history of diabetes. The serum phosphatase level is elevated in the disease and this enzyme is concerned with both bone formation and carbohydrate metabolism.

Insulin is not suggested as a cure or as a remedy to arrest the process, but for the alleviation of pain.

I should like to suggest that other clinicians attempt to verify this in their own cases of osteitis deformans.

964 Fisher Building,
Detroit 2, Michigan.

ROBERT C. MOEHLIG, M.D.
January 24, 1949.

lism. By Paul Fourman (by invitation), Edwin J. Kepler, Edward C. Reifenshtein, Jr., and Eleanor F. Dempsey (by invitation).

21. Changes in Urinary Steroids Produced by Sodium Deprivation and by Desoxycorticosterone Acetate Administration. By William H. Daughaday (by invitation) and Cyril M. MacBryde.

22. The Evaluation of Adrenocortical Function by Ascertaining the Response to a Single Injection of Adrenocorticotrophin. By H. W. McIntosh (by invitation), B. Singer (by invitation) and M. M. Hoffman.

23. The Level of Circulating Eosinophils as an Indicator of Adrenal Cortical Adequacy Following Major Surgery. By Marcel Roche (by invitation), A. Gorman Hills (by invitation) and George W. Thorn.

24. Is the Protein Metabolic Abnormality of Cushing's Syndrome Catabolic or Anti-Anabolic? By Sheldon Margen (by invitation), Laurence W. Kinsell, Erin K. Flanagan (by invitation), Lila E. Suiter (by invitation) and Elliot Rapaport (by invitation).

25. Hypokaliemic Alkalosis in Cushing's Syndrome. Observations on the Effects of Potassium Chloride and Testosterone Propionate Therapy. By Robert Teabeaut (by invitation), Frank L. Engel, and Haywood M. Taylor (by invitation).

26. The Mechanism of Action of Testosterone in the Therapy of Cushing's Syndrome. By Frederick C. Bartter (by invitation), Anne P. Forbes, William M. Jefferies, Evelyn L. Carroll (by invitation), and Fuller Albright.

SATURDAY, JUNE 4, 1949

9:00 a.m.—E. A. Doisy, presiding

27. Metabolism and Distribution of Thiourea in the Rat as measured with Radioactive Sulfur. By John Schulman, Jr. and Richard P. Keating (introduced by Rulon W. Rawson).

28. The Tracer Technique with Radioiodine I^{131} as a Potential Substitute for the Basal Metabolic Rate Determination in Routine Clinical Practice. By Sidney C. Werner.

29. The Distribution and Metabolism of Circulating Testosterone. By C. D. West and L. T. Samuels.

30. Pseudo-hypoparathyroidism: A Report of Two New Cases with Special Reference to the Epiphyseal Changes. By Harold Elrick (by invitation), Frederic C. Bartter (by invitation), Adney Sutphin (by invitation) and Fuller Albright.

31. Quantitative Measurements of the Growth of Axillary Hair as an Index of the Endocrine Status. By James B. Hamilton.

32. The Effects of Testosterone Propionate on the Peripheral Blood and Bone Marrow of Women with Advanced Carcinoma of the Breast. By Timothy R. Talbot, Jr. (by invitation) and George C. Escher.

33. Effects of Small Doses of Testosterone Propionate on Spermatogenesis. By Cleve Beller (by invitation) and Henry H. Turner.

34. Endocrine Factors in Gout: The Significance of Differences in Childhood and Adult Urate Metabolism. By William Q. Wolfson, David Krevsky, Rachmiel Levine (by invitation), Kinu Kadota (by invitation) and Clarence Cohn.

35. The Effect of Castration, of Unilateral Castration and of Pregnancy in Unilaterally Castrate Rats on the Ovary Transplanted into the Spleen. By Gerson R. Biskind and Morton S. Biskind.

36. The Occurrence of Conjugated Sulfates of Estrogens in Human Pregnancy Urine. By Herman Cohen (by invitation) and Robert W. Bates.

3. Studies on an Anti-Diuretic, Non Chloruretic Substance Extracted from Urines of Normal and Cirrhotic Subjects. By Elaine P. Ralli, Stephen Leslie (by invitation), George H. Stueck, Jr. (by invitation) Mary E. Dumm and Bertram Laken (by invitation).

4. A Method for the Assay of Prolactin in Human Urine. By Richard L. Coppedge (by invitation) and Albert Segaloff.

5. Thyrotrophic and Thyroid Hormone Assay of Normal and Pathologic Human Sera in the Stasis Tadpole. By S. A. D'Angelo, A. S. Gordon, K. E. Paschikis and A. Cantarow.

6. Estimation of Urinary Gonadotrophin of the Non-pregnant Human by the Mouse Uterine Weight and Ovarian Hyperemia Responses. By Charles W. Lloyd, Muriel Morley (by invitation), Kathryn Morrow (by invitation), Julia Lobotsky (by invitation) and Edward C. Hughes (by invitation).

7. Further Studies of Antigonadotrophin Formation in Man. By James H. Leatham and A. E. Rakoff.

8. The Evaluation of the Use of Anterior Pituitary Extract in the Treatment of Pituitary Dwarfism. By Joseph C. Edwards, Cecil M. Charles (by invitation) and Cyril M. MacBryde.

9. On the Inability of Adrenocorticotrophic Hormone or Epinephrine to Deplete the Ascorbic Acid of the Chick Adrenal. By Norman F. Boas (by invitation) and Joseph W. Jailer.

10. Regulation of Pituitary Adrenocorticotrophic Activity by Adrenal Cortical Hormones. By Chi-Ping Cheng (introduced by George Sayers).

11. Adequacy of Pituitary Adrenocorticotrophic Function in Nutritional Deficiencies. By George Sayers.

12. Effects of Prolonged Adrenal Cortical Stimulation upon Free and Esterified Serum Cholesterol in Normal Men. By Jerome W. Conn, and William C. Vogel (by invitation).

13. Possible Involvement of the Adrenal Cortex and Thyroid in Mobilization of Fat to the Liver. By Louis Levin.

FRIDAY, JUNE 3, 1949

2:00 p.m.—J. S. L. Browne, presiding

14. Stimulation of Nitrogen by Adrenal Cortical Extract during Insulin Hypoglycemia. By Frank L. Engel.

15. Renal Function in Normal and Adrenalectomized Rats following Saline or Adrenal Steroid Administration. By W. R. Boss (by invitation) James H. Birine and Robert Gaunt.

16. Adrenal Cortical Hormone in Blood. By K. E. Paschikis, A. Cantarow and D. Boyle (by invitation).

17. Urinary Corticoids. By Eleanor H. Venning, M. P. Ripstein (by invitation) and V. E. Kazmin (by invitation).

18. Studies on the Interrelationship of Adrenal and Thyroid Function. By Robert S. Reiss, Peter H. Forsham (by invitation) and George W. Thorn.

19. Clinical and Metabolic Changes in Addison's Disease Following the Administration of Compound E Acetate (11-dehydro, 17-hydroxy-corticosterone acetate). By P. H. Forsham (by invitation), L. L. Bennett (by invitation), M. Roche (by invitation), R. S. Reiss, A. Slessor (by invitation), E. B. Flink (by invitation) and G. W. Thorn.

20. Effect of a Single Dose of Desoxycorticosterone Acetate on Electrolyte Metabo-

54. Synthesis of Testosterone from Androstenedione-3, 17 by Testis Tissue. By Leo T. Samuels, Blaine H. Levedahl (by invitation), M. L. Helmreich (by invitation), and M. M. Pottner (by invitation).
55. The Role of the Adrenal Cortex in Some Somato-Sexual Aberrations in Infants and Children. By M. M. Melicow.
56. Effects of Compound E on Blood Ketone Bodies. By Leslie L. Bennett (by invitation), Alexander Slessor (by invitation) and George W. Thorn.
57. The Effect of Dietary Protein on the Ability of the Liver to Inactivate Estradiol in the Rat. By Joseph W. Jailer.
58. Pregnanediol Excretion in Cases of Blighted Ovum. By A. B. Abarbanel, Robert Hoyt (by invitation) and M. G. Levine (by invitation).
59. A Mechanism of Potassium Deficiency in Alkalosis. By Charles H. Burnett, Belton A. Burrows (by invitation) and Robert R. Commons (by invitation).
60. Effects of Hemopoietic Agents on Blood Formation in Hypophysectomized Rats. By Robert Gerstner (by invitation) and Albert S. Gordon.
61. The Relative Effectiveness of Desoxycorticosterone Acetate in Oil Solution and in Pellets Diluted with Cholesterol. By Albert Segaloff.
62. Adrenal Cortex Activity in Essential Hypertension. By Louis Tobian, Jr. and Harold Joseph (introduced by Carl A. Bunde).
63. The White Blood Cell Response of Rats to Adrenalectomy, Stress, and Pantothenic Acid. By Mary E. Dumm, Paul Roth (by invitation), Paul Ovando (by invitation) and Elaine P. Ralli.
64. Role of Emotional Stress in the Survival of Adrenalectomized Rats given Replacement Therapy. By Miguel R. Covian (introduced by Curt Richter).
65. The Androgenic Activity of New Esters of Testosterone. By A. J. Bergmann and Lloyd C. Miller (introduced by John S. L. Browne).
66. Effect of Androgen and Growth Hormone on the Rat's Os Penis. By Wm. R. Lyons, Edward Abernethy (by invitation) and Mark Grooper (by invitation).
67. The "Thiocyanate Space" and "Iodide Space" in the Thyroid Gland. By J. F. McClendon, William C. Foster (by invitation) and Emerson Reed (by invitation).
68. A Comparison of the 17-Ketosteroid Excretion of Cases of Cushing's Syndrome Due to Adrenal Tumor with Those Due to Hyperplasia (Hyperfunction). By Anne P. Forbes, Evelyn L. Carroll (by invitation) and Mary L. Wheeler (by invitation).
69. Sex Hormones and Staphylococcus Infections. By Manuel Villaverde.
70. The Problem of Allergy to Steroid Hormones. By George P. Heckel.
71. Intravenous Estrogen in Menometrorrhagia in the Human. By A. R. Abarbanel.
72. The Incidence of Cancer in Endocrine Case Histories. By J. K. Fancher and Jean Brooks (by invitation).
73. Renal Clearances in Patients with Cirrhosis of the Liver, with and without Ascites. By Stephen H. Leslie, Barbara Johnson (by invitation) and Elaine P. Ralli.
74. Porphyria Simulating Anorexia Nervosa. By Bernard A. Watson.
75. Hemosedimentation Test in Obesity. Aulo Pinto Viégas.
76. The Effects of Vitamin B, Thyroid, and Adrenal Alterations on the Amino Acid Oxidase Activity of Rat Liver and Kidney. By Samuel R. Tipton and Frances M. Colvin (by invitation).
77. The Problem of Endemic Goiter in Yunnan Province. By Isidor Greenwald.

37. Hormonal Factors Producing the Gametokinetic Response in the Male Frog (*Rana Pipiens*). By Robert B. Greenblatt, Sarah Clark (by invitation) and R. M. West (by invitation).

38. Action of Estrogens on Release of Luteinizing Hormone in Menopausal Women. By Arthur A. Hellbaum, J. W. Funnell (by invitation) and E. C. Keaty (by invitation).

39. The Hormonal Pattern in Pseudocyesis. By A. E. Rakoff and Paul H. Fried (by invitation).

40. Management of Threatened Abortion in the Human with Large Doses of Prethylstilbestrol. By A. B. Abarbanel.

SATURDAY, JUNE 4, 1949

2:00 p.m.—C. H. Best, presiding

41. A Hyperglycemic Factor Extracted from the Pancreas. 10 mins. By I. J. Pincus (introduced by A. E. Rakoff).

42. Studies in Carbohydrate Metabolism in Decerebrate Rats. 10 mins. By Evelyn Anderson and Webb Haymaker (by invitation).

43. Factors Affecting the Volume of the Islands of Langerhans. 15 mins. By R. E. Haist, Margaret Evans and B. Kinash (introduced by C. H. Best).

44. Studies on the Serum Potassium in Diabetic Acidosis. 15 mins. By Carl S. Nadler, Samuel Bellett and Mary Lanning (introduced by C. H. Best).

45. Pyruvic and Citric Acid Metabolism. 15 mins. By Max Miller and Ernest Bueding (introduced by C. H. Best).

46. Changes in Inorganic Serum Phosphorus during the Intravenous Glucose Tolerance Test as an Adjunct to the Diagnosis of Early Diabetes Mellitus. 15 mins. By Peter H. Forsham (by invitation), Marcel Roche (by invitation) and G. W. Thorn.

47. The Metabolism of Glucose and Galactose when Administered Simultaneously to Man. 10 mins. By G. C. Walsh (by invitation), M. M. Hoffman, H. T. McAlpine (by invitation) and E. H. Mason (by invitation).

48. Studies in Fat Metabolism. 1. Steroid Hormonal Effects Upon Blood Ketones and Other Intermediate Products of Fat and Protein Catabolism. 10 mins. By Laurance W. Kinsell, Sheldon Margen (by invitation), George D. Michaels (by invitation), Betty T. Signorotti (by invitation) and David P. McCallie (by invitation).

49. The Urinary Excretion of Corticosteroids in Diabetic Acidosis. 10 mins. By Janet W. McArthur, Randall G. Sprague and Harold L. Mason.

50. Steroid Diabetes Associated with Cushing's Syndrome and Excretion of 17-Hydroxycorticosterone (Compound F) in Urine; Metabolic Studies. 10 mins. By Randall G. Sprague, Alvin B. Hayles (by invitation), Harold L. Mason, Marschelle H. Power (by invitation) and Warren A. Bennett (by invitation).

51. Behavior of Electrolytes During Treatment of Diabetic Keto-Acidosis. 10 mins. By Jonas Weissberg, (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Dreker.

TO BE READ BY TITLE

52. Correlation of Vaginal Smears and Endometrial Biopsies in Normal Cycles and in Gynecic Disorders. By H. E. Nieburgs, Robert B. Greenblatt and S. Bamford (by invitation).

53. The Effect of Pteroylglutamic Acid Antagonists on the Response of the Reproductive Accessories of C57 Male Mice to Testosterone. By E. D. Goldsmith, H. M. Black (by invitation) and R. F. Nigrelli (by invitation).

Antithyroid Drugs. (Round Table Discussion)

E. B. Astwood, M.D., Boston, Massachusetts

Radioactive Iodine. (Round Table Discussion)

Mayo Soley, M.D., Iowa City, Iowa

Current Treatment of Hyperthyroidism. (Round Table Discussion)

John de J. Pemberton, M.D., Rochester, Minnesota

Treatment of Hyperthyroidism. (Round Table Discussion)

J. H. Means, M.D., Boston, Massachusetts

When is a Malignant Goiter Malignant?

Robertson Ward, M.D., San Francisco, California

Incidence of Carcinoma of the Thyroid in Nodular Goiter.

Warren Cole, M.D., Chicago, Illinois

What Thyroid Nodules Are to be Feared? A Basis for Deciding upon Surgical Exploration.

Oliver Cope, M.D., Boston, Massachusetts

Papillary Tumors of the Thyroid.

Shields Warren, M.D., Boston, Massachusetts

Non-encapsulated Sclerosing Tumor of the Thyroid.

J. Beach Hazard, M.D., and George Crile, Jr., M.D., Cleveland, Ohio

The Natural History of Thyroid Cancer.

Edgar L. Frazell, M.D., and Frank W. Foote, Jr., M.D., New York, New York

Lymphosarcoma of the Thyroid

Robert S. Dinsmore, M.D., Cleveland, Ohio

Surgical Treatment of Carcinoma of the Thyroid.

B. Marden Black, M.D., Rochester, Minnesota

Radio-Iodine Therapy of Metastatic Carcinoma of the Thyroid: A Six Year Progress Report.

S. M. Seidlin, M.D., Miss E. Oshry, I. Rossman and E. Siegel, New York, New York

Radio-Iodine in the Treatment of Metastatic Cancer of the Thyroid—Credits and Debits.

Jack B. Trunnell, M.D., Miss Ruth Hill, Benedict J. Duffy, Jr., M.D., Leonidas

Marinelli, M.D., Wendell Peacock and Rulon W. Rawson, New York, New York

Presidential Address—"Henry S. Plummer"

Arnold S. Jackson, M.D., Madison, Wisconsin

The Problem of Cancer of the Thyroid in a Non-Endemic Goiter Area

David H. Poer, M.D., Atlanta, Georgia

Some Goiter Problems Met With at West China Union University, Chengtu, Szechwan, China.

Charles H. Arnold, M.D., Lincoln, Nebraska

Cretinism.

Harry Colfer, M.D., Madison, Wisconsin

New Discoveries on the Innervation of the Larynx.

Brien T. King, M.D., and Ralph Gregg, M.D., Seattle, Washington

Metabolism Testing Under Anesthesia in Normal and Hyperthyroid Subjects.

Elmer C. Bartels, M.D., Boston, Massachusetts

Relationship of Lymphocytes and Fibrous Replacement to the Incidence of Postoperative Myxedema.

F. B. Whitesell, Jr., M.D., and B. Marden Black, M.D., Rochester, Minnesota

Struma Lymphomatosa.

T. C. Davison, M.D., and A. H. Letton, M.D., Atlanta, Georgia

The 1949 Meeting of the American Goiter Association

The annual meeting of the American Goiter Association will be held at the Loraine Hotel in Madison, Wisconsin, May 26 to 28, 1949. All members who have not made their hotel reservations are urged to do so immediately.

PROGRAM

Dietary Factors in the Pathogenesis of Simple Goiter.

Monte A. Greer, M.D., Martin G. Ettlinger, M.D., and E. B. Astwood, M.D.,
Boston, Massachusetts

Comparative Activity of Thiouracil and Other Antithyroid Compounds in the Rhesus Monkey.

D. A. McGinty, M.D., and M. L. Wilson, M.D., Detroit, Michigan

The Metabolic Fate of the Thyroid Hormone or its Derivatives.

W. T. Salter, M.D., New Haven, Connecticut

Metabolic Studies with I^{131} Labelled Thyroid Compounds.

Alexander Albert, M.D., and F. Raymond Keating, M.D., Rochester, Minnesota

The Calorigenic Properties of Tetrabromthyronine Tetrachlorthyronine as Assayed in Human Myxedema.

Jacob Lerman, M.D., Boston, Massachusetts

Thyroid Hormone-Like Properties of Tetrabromthyronine and Tetrachlorthyronine.

Charles E. Richards, M.D., Roscoe O. Brady, M.D. and Douglas S. Riggs, M.D.,
Boston, Massachusetts

The Antithyroxin Activity of Thyroxin Analogues.

Ruth Cortell, M.D., New York, New York

Thyroid-thyrotrophic Hormone Interaction in Body Fluids as Tested in the Starved Tadpole.

Savino A. D'Angelo, M.D., New York, New York

The Van Meter Prize Award Paper.

To be presented by winner of the Award

The Confessions of an Elderly Thyroidologist.

J. H. Means, M.D., Boston, Massachusetts

The Effects of Massive Doses of Potassium Iodide.

T. S. Danowski, M.D., Pittsburgh, Pennsylvania

The Thyroxin-like Action of Elemental Iodine

Samuel Dvoskin, M.D., New York, New York

The Functional Capacity of Various Types of Thyroid Carcinoma as Revealed by the Autoradiographic Demonstration of Radioactive Iodine.

Patrick J. Fitzgerald, M.D., New York, New York

A Method for the Preoperative Estimation of Function of Thyroid Tumors: Its Significance in Diagnosis and Treatment.

Brown M. Dobyns, M.D., and Bengt N. Skanse, M.D., Boston, Massachusetts

Thyroidectomy. (Round Table Discussion)

Richard B. Cattell, M.D., Boston, Massachusetts

Abstracts of

CURRENT ENDOCRINE LITERATURE

Editor: ROY HERTZ. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS

PANCREAS

HILES, C.: Gastric secretory response in hypoglycemia as produced during insulin shock therapy, *Am. J. M. Sci.* 214: 667-672, 1947.

The purpose of this report is to emphasize that insulin shock therapy as employed in the treatment of mental disease may cause serious side effects. The study reveals the increase in gastric acidity in patients with schizophrenia when given insulin shock therapy.

After a limited review of the literature, five cases are reported including graphs on the following data: sugar, total acid, free acid, blood, coma and bile.

The cases illustrate the effect of hypoglycemia upon gastric secretion. In these 5 cases of schizophrenia given insulin shock treatment, a definite increase in the acid content of the stomach occurred during the periods of hypoglycemia and since it is believed that gastric hyperacidity is one of the contributory factors in the production of peptic ulcer, thorough gastro-intestinal studies should be completed in such patients before giving insulin. Measures to counteract or nullify the gastric secretory response to hypoglycemia should also be instituted.—*E.C.R., Jr.*

LEECH, R. S., and FORD, N. W.: A simple bedside method for the estimation of blood sugar, *J. Lab. & Clin. Med.* 33: 644-650, 1948.

A simple method for rapid estimation of blood sugar, based on the reduction by glucose of dinitrosalicylic acid, was designed primarily for the physician or nurse with a minimum of time and equipment and limited laboratory facilities. In its present form, the method is not suitable for estimating very low blood sugar values; between 50 and 75 mg. per cent it is possible to make fairly good approximations. Comparison of the results obtained on 60 blood samples, using the method described, with those of other standard methods, shows that they check closely with the determinations as carried out by the Folin-Wu macromethod and the Folin-Malmros or the rapid Hagedorn-Halstrom-Jensen micromethods.—*T.H. McG.*

MILLARD, E. B., and ROOT, H. F.: Degenerative vascular lesions and diabetes mellitus, *Am. J. Digest. Dis.* 15: 41-51, 1948.

The authors analyzed the autopsies of 110 diabetic patients who had been studied at the New England Deaconess Hospital from 6 months to 29 years and whose deaths occurred between 1940 and 1945. The average duration of life after the onset of diabetes was 9.8 years. The average life expectancy in this group was 44 per cent of the general popula-

Total Thyroidectomy: A Supplemental Report of 280 Cases of Diffuse Toxic Goiter Treated by this Method.

A. C. Scott, Jr., M.D., and P. M. Ramey, M.D., Temple, Texas

Pneumothorax Following Thyroidectomy (A Report of Two Cases)

Lindon Seed, M.D., Chicago, Illinois

A Simplified Clinical Method for the Determination of Blood Iodine.

Arthur C. Connor, M.D., Roy E. Swenson, M.D., George M. Curtis, M.D., Columbus, Ohio

Treatment of Recurrent Hyperthyroidism.

William S. Reveno, M.D., Detroit, Michigan

The 1949 Annual Meeting of the American Diabetes Association

CHALFONTE-HADDON HALL,

ATLANTIC CITY, N. J.

SATURDAY AFTERNOON, JUNE 4;

SUNDAY MORNING AND AFTERNOON, JUNE 5.

BANQUET, SATURDAY NIGHT.

Please send reservations for the banquet now to this office. Wives of members are welcome. Dinner subscriptions—\$6.00—*Payable when you register at the meeting.*

LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the A.A.A.S. will hold its 1949 meeting at the Forest Hills Hotel, Franconia, New Hampshire, September 12 through 17.

Attendance at this Conference is limited by the accommodation available at the hotel but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee on Arrangements consists of R. W. Bates, E. R. Squibb & Sons; R. D. H. Heard, McGill University; A. D. Odell, Charles E. Frosst & Co.; E. C. Reifenshtein, Jr., Sloan-Kettering Institute; A. White, School of Medicine, University of California at Los Angeles; and G. Pincus, Chairman, The Worcester Foundation for Experimental Biology.

Applications for attendance at the Conference should be sent to the Chairman at 222 Maple Avenue, Shrewsbury, Massachusetts. Applications to be considered by the Committee must be received by June 6, 1949.

stilbestrol and progesterone were given to the patients in varying amounts. Compared to a group reported previously by the same authors, in which no hormones were employed, the current group exhibited an increase in fetal survival from 60 to 76.9 per cent—*J.M.*

REED, J. A.: Statistical analysis of deaths from diabetes in the District of Columbia for a period of 42 years—1903–1944 inclusive. Preliminary report and analysis: a comparison of two five-year periods—1903–1907 and 1940–1944, *Am. J. Digest. Dis.* 15: 233–238, 1948.

The author's analysis was based on data obtained from the death certificates, signed by the attending physician, which were filed with the Division of Vital Statistics of the Health Department of the District of Columbia. The cases were divided into two groups: 1) those in which diabetes was the immediate or primary cause of death, and 2) those in which diabetes was a secondary or contributing cause of death. The two five-year periods represent intervals approximately 20 years before and after the institution of insulin therapy. Although the population of the area increased $2\frac{2}{3}$ times between 1903 and 1944, the deaths due to diabetes increased fivefold. In the 1903–1907 period, 77.5 per cent of the deaths of diabetics were due primarily to diabetes; in the 1940–1944 period, this figure dropped to 27.5 per cent. The author felt that this significant change was due to better medical management of diabetes through diet and insulin. With improved therapy, patients lived longer and other primary causes of death came to the fore. Whereas males constituted 53 per cent of diabetics who died in the first period, they constituted only 37 per cent in the second period. Between 1903 and 1907, 70 per cent of the population was white and 30 per cent was colored: ninety-two per cent of the diabetic dead were white and 8 per cent were colored. Although the population composition between 1940 and 1944 remained the same as in the earlier period, the whites accounted for 71 per cent of the deaths and the colored for 29 per cent of the deaths in the later period. When diabetes was the primary cause of death in those cases complicated by gangrene, the incidence of these cases was 8 per cent in the 1903–1907 period and 19 per cent in the 1940–1944 interval. Similarly where coma and/or acidosis complicated diabetes, the incidence rose from 49 per cent to 55 per cent. However, when these complications were present in the cases which included deaths primarily due to diabetes as well as deaths in which diabetes was a contributing factor, the incidence fell from 40 per cent to 17 per cent. The average age at death of all diabetics in both periods was the same (59.7 years). The average age of those patients in which death was primarily due to diabetes was 50 years in the period 1903–1907 and 54.6 years in the period 1940–1944. It was pointed out that the percentage of cases treated in hospitals rose from 19 per cent to 53 per cent. Where diabetes was a contributing cause of death, the incidence of cardiovascular-renal diseases remained unchanged in the two periods.—*H.S.G.*

SCHWARTZMAN, J.; CRUSIUS, M. E., and BEIRNE, D. P.: Diabetes mellitus in infants under one year of age: report of a case and review of the literature, *Am. J. Dis. Child.* 74: 587–606 (Nov.) 1947.

This is a general review of the subject wherein 57 cases of diabetes mellitus in infants under one year reported in the literature to date are presented in tabular form (from as early as 1850) giving the author and reference, age at onset, sex, family history of dia-

tion average. Among the younger patients (less than 33 years of age) who died within six years of the development of diabetes, none showed arteriosclerosis. When diabetes had been present in this group for more than 14 years, all patients showed severe degenerative vascular lesions (coronary atheromata, intercapillary glomerulosclerosis, pyelonephritis, nephrosclerosis and chronic glomerulonephritis), and hypertension. Among the 100 patients analyzed, the cause of death was directly attributable to diabetes in 5 instances; diabetes was a "critical factor" in the deaths of 85 patients. Diabetic coma accounted for 3 per cent of the deaths. Thirty-six per cent of the deaths were due to degenerative heart disease. Sixty-eight per cent of the hearts examined showed some degree of coronary artery narrowing. Forty-one per cent of the patients had no palpable dorsalis pedis artery pulsations before death. Degenerative vascular lesions in the kidneys were seen in 52 per cent of the cases. Abnormalities in the pancreas were noted in 79 per cent of the patients. Hypertension was present in 59 per cent of the people examined.

The authors felt that premature vascular lesions were postponed in those cases in which diabetic coma, acidosis, and hypoglycemic reactions were avoided by careful medical management.—*H.S.G.*

MIRSKY, I. A.; PODORE, C. J.; WACHMAN, J., and BROH-KAHN, R. H.: The urinary excretion of insulin by normal and diabetic subjects, *J. Clin. Investigation* 27: 515-519, 1948.

The problem of urinary excretion of insulin has been reinvestigated in an effort to clarify previous conflicting reports. A procedure was devised for assaying small quantities of insulin which had been added to urine before and after lyophilization. Results of assays in normal and diabetic subjects showed statistically significant differences; the average daily excretion of insulin by normal subjects was 0.16 ± 0.04 units, that of mildly diabetic subjects who did not require insulin 0.07 ± 0.03 units. Of even greater interest was the finding that both normal and diabetic subjects excreted only infinitesimal fractions of large doses of exogenous insulin. Two diabetic patients with "insulin resistance" excreted less than did nondiabetic subjects who had received large amounts of exogenous insulin. One of these patients, requiring 240 units per day, excreted 0.2 units daily; the other, with a daily requirement of 350 units, excreted 0.3 units. In comparison to these figures, 0.6 units were excreted by the nondiabetic subject after injection of 400 units. The assumption that insulin undergoes a rapid destruction within the body is indicated by the data from these studies.—*T.H.McG.*

MOSENTHAL, H. O.: Management of diabetes mellitus; an analysis of present day methods of treatment, *Ann. Int. Med.* 29: 79-90, 1948.

This is a review article which proposes a three point plan in the management of diabetes. The value of normal blood sugar concentrations and avoidance of glycosuria is recognized, but the avoidance of hypoglycemia is the paramount consideration. Adjustment of food intake, diet calculations, auto-urine analysis, and medical supervision is fundamental. Attention should be given to the conservation of qualitative nutrition as measured by hemoglobin, red blood cell count, serum protein levels, and avoidance of obesity—*C.D.D.*

PALMER, L. J.; CRAMPTON, J. H., and BARNES, R. H.: Pregnancy in the diabetic, *West. J. Surg.* 56: 175-177, 1948.

Thirty-nine pregnancies in 37 diabetic women are the subject of this study. Diethyl-

ZIMMERMANN, B., and DONOVAN, T. J.: Hyperglycemic effect of insulin, *Am. J. Physiol.* 153: 197-204, 1948.

Commercial insulin possesses a hyperglycemic property which is not destroyed when the hypoglycemic effect is inactivated. Amorphous and crystalline insulin inactivated with cysteine showed equal hyperglycemic effects in dogs with respect to magnitude and duration. In pancreatectomized dogs not treated with insulin the hyperglycemic response was small and the blood ketone bodies rose simultaneously with the blood sugar following administration of both natural and inactivated insulin. The inactivated insulin does not protect mice against insulin convulsions. It is concluded that the hyperglycemic principle acts on the hepatic glycogen reserves but does not neutralize the hypoglycemic effect of insulin.—A.E.M.

GENERAL

DE VRIES, A., and ALEXANDER, B.: Studies on amino acid metabolism. II.

Blood glycine and total amino acids in various pathological conditions, with observations on the effects of intravenously administered glycine, *J. Clin. Investigation* 27: 655-664, 1948.

The glycine and total α -amino N levels were studied in several pathological conditions, including a group of endocrine diseases. All glycine values were low in a hypermetabolic patient with a myopathy simulating myasthenia gravis. Elevated values, persisting for at least one month after ablation of the hypophysis, were observed in an acromegalic patient. Blood amino N was normal in the hypermetabolic subjects tested. Glycine showed a tendency to elevation in 5 cases of hypometabolism; in only one, a cretin, was the α -amino N also increased. Plasma amino N, originally low in a patient with hypometabolism and nutritional hypoproteinemia, rose steadily, while plasma glycine values increased suddenly from low normal and remained elevated. In one patient with hypothyroidism and in one with acromegaly and hypermetabolism, the glycine tolerance test showed very high plasma and red cell glycine following injection, with an abnormally slow decline; high fasting blood glycine was also noted in these two subjects. A marked rise in plasma glycine after injection, but with an essentially normal subsequent decline, occurred in one patient with hypometabolism and nutritional hypoproteinemia. Low normal plasma glycine was demonstrated in two insulin-treated diabetics, and elevated whole blood and red cell glycine in a case of Addison's disease with scleroderma. T.H.McG.

FABRYHART, M., and PACELLA, B. L.: Association of spontaneous hypocalcemia and electrocerebral dysfunction, *Arch. Int. Med.* 81: 184-202, 1948.

Eight patients presenting symptoms usually observed in psychoneurosis, spontaneous hypoglycemia or hypocalcemia, were studied. Oral glucose tolerance tests, serum calcium determinations, and electroencephalograms were done. The authors stressed the similarity of symptoms in all three conditions. A direct correlation between concentration of glucose in the blood and clinical symptoms was not found, and in the opinion of the authors an abnormally low curve for glucose tolerance is an important and sufficient diagnostic criterion when the clinical picture is present. The electroencephalographic changes consisted for the most part of slow type alpha rhythm and random 6 to 7 cycles per sec-

betes, symptoms, laboratory findings (glycosuria, blood sugar), outcome, and autopsy observations. To these, a case of an 8-month-old girl with diabetes mellitus is added.

Heredity, infections and disorders of the central nervous system were the main etiologic factors, in the order given. Early symptoms may be loss of weight, dry or sensitive skin, irritability and/or crystalline deposits on the diapers. Common complications were observed to be: dermatologic conditions, gangrene, cataract, infections of the respiratory tract, acidosis and coma. The mortality rate of 50 cases was calculated, these having been divided into two periods—the preinsulin period extending to 1924, and the insulin period, from 1925 to the present. The figures were 88.5% and 50% respectively. The commonest observations at autopsy were atrophy of the pancreas, with decrease in size and number of the islands of Langerhans, and fatty degeneration of the liver.

Treatment is based on a diet approaching the normal one, supplemented with insulin and vitamins, as needed. Furthermore, it is evident that the earlier the onset of diabetes mellitus, especially if the patient is under 3 months of age, the worse the prognosis; and the longer the disease has persisted unrecognized and without proper treatment, the poorer the outlook.

It is concluded, that from the standpoint of the formulation of a prophylactic program, early detection of the disease is most important, so that proper care may be instituted. The following procedures are recommended: 1) in all cases with a familial history of diabetes: routine dextrose tolerance tests every two months for the first year and every six months thereafter; 2) during infections: daily urinalysis, with sugar tolerance tests at the onset and at the end of the illness; 3) after any infection: routine urinalysis every two weeks for a period of 3 months, followed by a dextrose tolerance test at the end of that time.—*E.C.R., Jr.*

TUYNMAN, P. E., and WILHELM, S. K.: Potassium deficiency associated with diabetic acidosis, *Ann. Int. Med.* 29: 356-361, 1948.

A case report is presented of a patient who developed respiratory distress and weakness seven and a half hours after treatment for diabetic acidosis had begun. The respiratory embarrassment was not relieved by oxygen but was rapidly relieved by oral potassium chloride.—*J.M.*

VERZAR, F., and WENNER, V.: The influence *in vitro* of desoxycorticosterone on glycogen formation in muscle, *Biochem. J.* 42: 35-41, 1948.

Surviving rat diaphragm synthesizes glycogen in Ringer solution containing glucose. This process is accelerated by insulin and inhibited by desoxycorticosterone. The effect of 1 unit of insulin is completely inhibited by 5 to 10 mg. of the latter hormone.—*H.L.M.*

WILKERSON, H. L. C., and HEFTMANN, E.: Screening method for blood glucose, *J. Lab. & Clin. Med.* 33: 236-238 (Feb.) 1948.

A simple and inexpensive method for blood glucose has been described. It is a modification of Hagedorn's potassium ferrocyanide method. The method, using 0.1 ml. of blood and reagents in tablet form, is so designed as to demonstrate the presence of sugar within 5 minutes, only if the concentration is above an arbitrarily selected critical level. In 50 cases examined by this method 25 blood samples below 170 mg. per cent glucose gave a negative result, 21 samples above 180 mg. per cent a positive result and four samples between 170 and 180 mg. per cent either a positive or a negative result.—*T.H.McG.*

servations on the ketosteroid content of urine from patients with prostatic carcinoma and adenoma, *Cancer Research* 7: 534-536, 1947.

Determination by the method of Holtorff and Koch of 17-ketosteroids in 200 24-hour specimens of urine from 32 cases of prostatic carcinoma and 39 cases of benign prostatic adenoma revealed no characteristic alteration of androgen excretion in the prostatic carcinoma subjects. The values were characteristically low as would be expected for the age group tested. There was a definite correlation between specific gravity and total urinary volume suggesting some relationship between renal function and ketosteroid excretion.—*R.H.*

MERCHANTE, FERMIN RAUL: Pregnancy test with the reaction of Galli Mainini, *Semana méd.* 55: 49-53, 1948.

In this test the urine of the patient is injected into the dorsal lymph sac of the male toad and the appearance of spermatozoa is observed in the urine collected from the bladder. Two toads are used per test and the observation is carried out over 3 to 24 hours although a positive reaction may be observed as early as after 100 minutes. A maximum of 10 cc. of urine is used. In 266 tests correct negative results were recorded in 100 per cent, and correct positive results in 98 per cent of the cases.—*A.E.M.*

PINCUS, J. B.; NATELSON, S., and LUGOVY, J. K.: Response of citric acid levels to oral administration of glucose. II. Abnormalities observed in the diabetic and convulsive state, *J. Clin. Investigation* 27: 450-453, 1948.

Part I of this investigation demonstrated *in vivo* in man that the normal response to active carbohydrate metabolism, as in the postabsorptive period for glucose or after injection of insulin, was a lowering of blood citric acid levels. Most of the diabetics studied in this investigation showed no apparent deviation from the normal citric acid level response curve when glucose was administered orally. However, certain diabetic patients with neurologic symptoms who were difficult to control with insulin therapy showed abnormalities in their citric acid response by a rapidly rising level, or by a sharp rise for the first hour of the test period followed by a lowering to the minimum level.—*T.H.McG.*

STUART, HAROLD C.: Physical growth during adolescence, *Am. J. Dis. Child.* 74: 495-502 (Oct.) 1947.

The author divides the progress of growth from conception to full maturity into 3 major phases: the first and last being characterized by cycles of acceleration followed by deceleration in growth rates, and both accompanied by striking developmental changes. The middle phase is one of relatively steady, moderate progress in both growth and maturation. The last of these three phases is rather loosely called adolescence and preferably called pubescence which is separated from childhood by the preparatory or transitional stage, prepubescence, and from full maturity by a terminal stage, postpubescence.

During prepubescence the rate of body growth begins to accelerate, the sex organs grow more rapidly and certain endocrine glands increase their activity. However, much of the growth of the sex organs takes place during the postpubescent years, after the person has attained approximate adult size and after he appears to have reached ma-

and potentials. These changes were considered to reflect a decreased threshold for the development of epileptiform clinical reactions, and not as indicative of epilepsy or a convulsive disorder. Treatment consists of a maintenance diet high in protein with supplementary feedings, phenobarbital and belladonna. Calcium salts were used orally and parenterally in those patients who had associated hypoglycemia.—*C.D.D.*

GALLI MAININI, C.: Pregnancy test using the male batrachia, *J.A.M.A.* 138: 121 (Sept. 11) 1948.

The author has now thoroughly tested the method he described in 1947 which makes use of the male toad for the assay of chorionic gonadotropin. In his hands the test is remarkably simple and reliable. The injection of 10 cc. of pregnancy urine into the dorsal lymph sac of the male toad causes the appearance of spermatozoa in the urine within one to three hours. No preparation of the urine is required. The results are read by the direct examination under the microscope of a few drops of urine removed from the cloaca. Two thousand and thirty tests were done. In the first to sixth months of pregnancy 99% of urines gave a positive result, in the last trimester 92% did. No false positive test occurred in 623 assays of urine from a variety of nonpregnant cases.—*A.P.F.*

GOLDZIEHER, JOSEPH W.: A new colorimetric method for the determination of pregnandiol, *J. Lab. & Clin. Med.* 33: 251-253 (Feb.) 1948.

The author describes a colorimetric method for determining pregnandiol which is as sensitive as the sulfuric acid method and yet does not develop adventitious color. The method is based on the interaction of pregnandiol with acetyl chloride and zinc chloride in glacial acetic acid solution. The color obtained is stable and at room temperature, it remains constant for at least two and a half hours. With the use of a standard, containing 0.5 mg. of pregnandiol, the method was found to be accurate within ± 4 per cent.—*T.H.McG.*

LEVIN, E.; KIRSNER, J. B., and PALMER, W. L.: Preliminary observations on histamine and insulin stimulated gastric secretion during the injection of an enterogastrone concentrate in man, *Gastroenterology* 10: 274-280, 1948.

The effect of moderately large quantities of an enterogastrone concentrate upon the gastric secretory response to histamine and to insulin was studied in 15 patients with peptic ulcer. Intramuscular injection of 1000 mg. of the concentrate was followed in one of three patients by a decrease in the gastric secretory response to a single standard dose of histamine; in the one patient studied, the maximum free acidity was not affected by the administration of 3000 mg. of enterogastrone. In seven patients, intramuscular administration of 400 to 2000 mg. of enterogastrone did not alter the gastric secretory response to the repeated injection of small quantities of histamine. Insulin-stimulated gastric secretion in three patients was not altered by intramuscular injection of 1000 mg. of enterogastrone; in one, it was possibly delayed by 2000 mg. and may have been suppressed by 3000 mg. in one. Further observations with more potent preparations in a larger series of cases will be necessary before definite conclusions can be drawn from the last two experiments.—*T.H.McG.*

McHENRY, E. W.; SEMMONS, E. M.; PEARSE, R., and MEYER, E. G.: Ob-

given 35,000 mg. of testosterone propionate over a two-year period with apparent prolongation of life in comfort.—*R.A.C.*

ZONDEK, B., and BRZEZINSKI, A.: Inactivation of oestrogenic hormone by women with vitamin B deficiency, *J. Obst. & Gynaec. Brit. Emp.* 55: 273-280, 1948.

Fourteen cases with marked evidence of vitamin B-complex deficiency were studied. No change in estrogen inactivation was found and estrogen titers in blood and urine were normal as well as vaginal smears and endometrial biopsies. It was concluded that the vitamin was not an essential factor in the estrogen-inactivating mechanism.—*R.A.C.*

ZONDEK, B.; SULMAN, F., and BLACK, R.: Hormonal test for fetal death in disturbed pregnancy, *J.A.M.A.* 136: 965 (Apr. 10) 1948.

Urinary gonadotropin is titrated by the rat ovary hyperemia test described by the authors in 1945. The HU (hyperemia unit) is established for each strain of rat by correlation with estrus units. The technique of the test is reviewed. In disturbed pregnancy a titration is made on four rats injected with 4, 2, 1, and 0.2 cc. of urine which corresponds to titers of 250, 500, 1000 and 5000 HU per liter. Less than 1000 HU indicates probable, and less than 500, certain fetal death in the first two trimesters. During the peak of gonadotropin excretion, between the fortieth and eightieth days after implantation, the normal excretion is about 200,000 HU per liter. A level of 5000 units at this time is consistent with fetal death. Repetition of the test after a week to determine whether the titer is rising or falling assists in predicting the outcome of the pregnancy. Five hundred cases of disturbed pregnancy were studied.—*A.P.F.*



turity in most other respects. The gradual transition from one stage to another and the great individual variability in respect to age make it impossible to delimit precisely the early and late stages of adolescent development in terms of chronologic age, but roughly each stage may be thought of as lasting 2 to 3 years in most persons.

Primary attention in this discussion is devoted to the changes with growth, which are characteristic of the period referred to as pubescence. The principal characteristics of the pubescent growth of the skeleton, including the muscles and the overlying soft tissues are listed and a table showing the common, or characteristic, differences between early and late-maturing persons is presented.—*E.C.R.*

SWANK, R. L., and BERGNER, G. E.: A study of the human myogram. A study of normals, and of patients with Addison's disease, thyrotoxicosis and progressive muscular atrophy, *J. Clin. Investigation* 27: 24-33 (Jan.) 1948.

A method of myography commonly used in animals was employed in a group of human subjects to record alterations in muscle function. Slight changes in the myogram, correlated with fatigue, were observed in normal subjects. An early dip in the myogram occurred in patients with Addison's disease who exhibited marked weakness. Adequate treatment of these patients with desoxycorticosterone was followed by reversal of these changes toward normal. In the patients with Addison's disease and in two with thyrotoxic myopathy, an absence of potentiation of the muscular contractions normally present during repeated stimulation, was observed. Severe and generalized weakness accompanied this alteration in the myogram, apparently a manifestation of the abnormal metabolic state in the muscles of patients with Addison's disease. Neither hypoxemia nor hypoglycemia of a severe grade per se produced any altered responses in types of tests for muscular function which were here employed. Marked variation in the height of muscular contraction was noted in patients with progressive muscular atrophy.—*T.H.McG.*

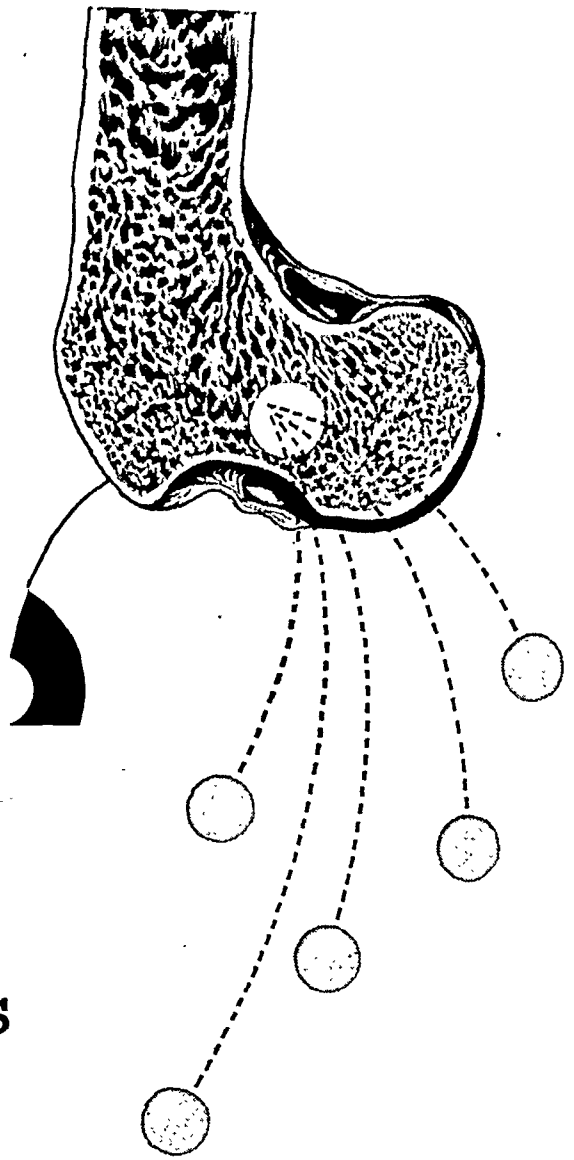
ULLMANN, T. D., and SCHORR, S.: Renal dwarfism with hyperparathyroidism in a case of congenital malformation of the kidneys, *Ann. Int. Med.* 29: 715-730, 1948.

A case of renal dwarfism in a 14-year-old girl, characterized by stunted growth (without manifestations of rickets), infantilism, bilateral congenital dilatation of the renal pelvis and ureters with atrophy of the renal parenchyma and interstitial nephritis, parathyroid hypertrophy and slight osteoporosis is presented. The congenital nature of the kidney disorder, the retardation of growth since early infancy and the presence of similar kidney disorders and subnormal stature in two other female members of the same family are thought to justify an application of the continental theory of "multiple congenital malformations" to the early nonuremic phase of this case. The Anglo-American theory, which maintains a causal relationship between renal insufficiency and skeletal involvement, is invoked to explain only the late manifestations of the patient's disease.—*J.M.*

WYATT, J.: Testosterone propionate in inoperable carcinoma, *J. Obst. & Gynaec. Brit. Emp.* 55: 53-54, 1948.

A case of squamous celled carcinoma of the uterus in a woman aged 57 years. She was

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respiratory quotient. The 11-oxysteroids also counteract the hypoglycemic action of insulin (4, 5, 6, 7).

There is some conflict of evidence as to whether the whole of the diabetogenic action of pituitary extract is obtained by inducing hyperplasia and hyperfunction of the adrenal cortex. Houssay (8), the pioneer demonstrator of the diabetogenic action of pituitary extracts in the hypophysectomized depancreatized animal (Houssay animal) found that bilateral adrenalectomy did not prevent the diabetogenic action but hepatectomy did. Long and Lukens (9) however, found that all the significant changes in carbohydrate metabolism that occur in the Houssay animal are essentially the same as those met with in the pancreatectomized-adrenalectomized animal (Long-Lukens animal) and that the diabetogenic effects of the anterior pituitary gland were no longer obtained if both adrenal glands were completely removed. With 11-oxysteroids they could also re-establish diabetes in the adrenalectomized-pancreatectomized animal. The conflicting evidence is probably due to different species of animals being used but it seems certain that part of the diabetogenic action of pituitary extracts in mammals is mediated via the adrenal cortex (4, 9).

CASE REPORTS

Case 1. This case was described in my 1932 paper (1). The essential features were as follows: A boy stopped growing at the age of 13 and showed lassitude and weakness. At the age of 16 he presented a picture of infantilism, but pigmentation of his skin led to the correct diagnosis of Addison's disease. At this time, April 10, 1931, fasting blood sugar values were 53 mg. and 69 mg. per cent. Beginning April 18, a dose of 10 cc. of adrenal cortical extract was injected daily. Four weeks later the blood sugar reached diabetic levels of 220 mg., 250 mg., and 360 mg. per cent but the patient showed extreme sensitivity to small doses of insulin, with hypoglycemic reactions. He died on June 3, 1931, in Addisonian crisis, and autopsy showed atrophy of the suprarenals and of the pancreas. It was concluded that both pathologic lesions occurred at the same time and were of the same origin, and that the diabetes was initially obscured by the adrenal insufficiency. It would appear that the therapy with adrenal cortical extract was a factor in revealing the diabetes.

Case 2. D. C., a 37-year-old male, developed diabetes mellitus in 1921 at the age of 11, Addison's disease in 1942 at the age of 32, and died in 1947 at the age of 37 from acute adrenal insufficiency. For the first two years after the onset of the diabetes he received dietetic treatment, but in 1923 insulin was commenced. There are no quantitative details of early dosage, but it is known that in December 1941 he was receiving 36 units of soluble insulin twice daily, with a carbohydrate intake of 250 grams daily, and that during the the next few months, owing to attacks of hypoglycemia, the insulin was progressively reduced to 24 units twice daily. In spite of this apparent improvement in his carbohydrate tolerance, the patient was easily tired and did not feel well. In February, 1942, nausea and vomiting developed, in spite of a normal blood sugar and no ketosis, and about the same time some pigmentation of the face and neck appeared. He had been attending Dr. R. D. Lawrence's diabetic clinic at King's College Hospital, and was admitted as an In-Patient on March 13, 1942, in a collapsed and dehydrated state. His

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ADDISON'S DISEASE AND DIABETES MELLITUS IN THREE PATIENTS

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IN 1932 (1) the writer described a case of diabetes mellitus developing in a lad of 16 years of age with pre-existing Addison's disease. Autopsy revealed atrophy of the cortex of both adrenal glands and of the islets of Langerhans. In the present paper 2 further cases of the coexistence of Addison's disease and diabetes mellitus are recorded, one coming to autopsy; and a résumé is given of 15 other cases recorded in the literature, in which autopsies were recorded, and 6 additional cases without autopsies.

PHYSIOLOGY

Adrenalectomy results in lowering of blood sugar, particularly after starvation, and in the terminal phase death from hypoglycemia. In some species, *e.g.* marmots, other effects on carbohydrate metabolism are depletion of liver glycogen and to a lesser extent of muscle glycogen, increase of blood lactates and increase in the rate of metabolism of glucose (2, 3, 4). These effects are reversed or prevented by the administration of an adrenalin-free extract of the adrenal cortex, or by any of the 11-oxysteroids that have been isolated from the adrenal cortex, *e.g.* corticosterone, 11-dehydrocorticosterone, and 11-dehydro 17-hydroxycorticosterone (Kendall's compound E). This group of 11-oxysteroids also has a diabetogenic action as has been demonstrated in the partially depancreatized or phloridzinized rat; and the glucose-nitrogen ratio of 3.6 to 1.0 indicates that the source of the extra glucose is protein. There is also a deposition of hepatic glycogen and a depression of glucose oxidation, as shown by lowering of the

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good condition. On November 30, 1943, I implanted subcutaneously 600 mg. of desoxycorticosterone (6 tablets) and on December 17, 1943, he felt well. His serum sodium was 348, potassium 18.3, chloride 376, and urea 29 mg. per cent; alkali reserve (CO_2) 65 volumes per cent; blood sugar 80 mg. per cent; and B.P. 125/85. In February, 1944, he was still well. His B.P. was 140/90 and his pulse 74. Pigmentation was very slight and his weight was 120 pounds (before illness, 126 pounds). On April 5, 1944, his clinical condition was deteriorating, and he complained of weakness, nausea and hiccups. The addition of methyltestosterone, 5 mg. three times daily by mouth, had no effect. On April 26, 5 mg. of desoxycorticosterone was injected twice daily, and he immediately felt better. A few days later the serum sodium was 333, chloride 341, potassium 20, and urea 35 mg. per cent; and alkali reserve (CO_2) 61 volumes per cent. It was estimated that the implantation had been effective for some six months, and on May 26 I implanted 800 mg. of desoxycorticosterone with beneficial results. The patient was not seen for a year, but he called again on May 8, 1945. He had been well until March, 1945, when he had recommenced injections of desoxycorticosterone, 5 mg. twice daily. His insulin has been progressively reduced by himself to 2 units in the morning and 6 units in the evening, and his carbohydrate increased to 300 Gm. daily, because of recurrent hypoglycemic attacks. On May 22, 1945, I implanted 900 mg. of desoxycorticosterone acetate. There is here a gap in the notes on the attendances but on April 9, 1946, I implanted 800 mg. of desoxycorticosterone. Edema of the face developed in the next week but subsided after giving potassium citrate by mouth for a few days. On June 5, 1946, the patient was admitted to King's College Hospital under the care of Dr. Lawrence, with vomiting and malaise. His blood pressure was 140/90, temperature 105° , pulse 136, and respiration 24. His urine contained no sugar but much acetone, and his blood sugar was 120 mg. per cent. His dose of insulin was 2 units in the morning and 4 units in the evening. The blood chemistry suggested slight overdosage with desoxycorticosterone: sodium 354, potassium 15.7, chloride 350 and urea 24 mg. per cent. He was better without treatment within a few days and was discharged from the hospital.

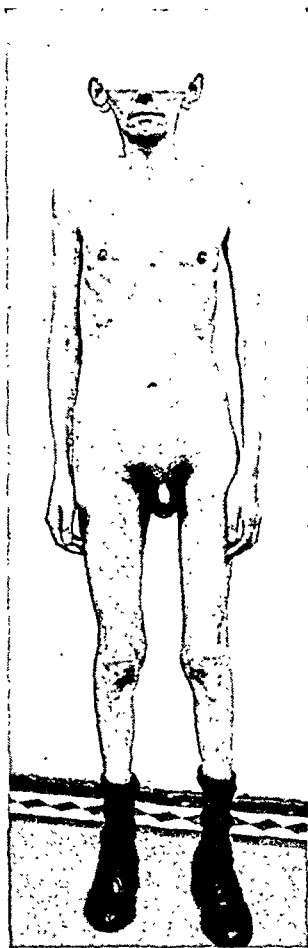


FIG. 1. Case 2. Photograph of patient taken three weeks before death, showing characteristic degree of emaciation in spite of biochemically controlled therapy.

better and stronger, but on December 10 his wife noticed some puffiness around the eyes. That evening he complained of headache, weakness, shivering and perspiration. The next morning, he was incontinent and semicomatose and edema of the face was more marked. His wife then telephoned me and he was admitted to Willesden General Hospital. His pulse was hardly palpable and the blood pressure difficult to record, (?) 100/60. There were no crepitations in the lungs. He was conscious but very deaf. His blood sugar was 135 mg. per cent, and blood taken for electrolyte assay was reported a few days

On November 25, 1947, I implanted 700 mg. of desoxycorticosterone and 400 mg. of testosterone, the latter because of impotence which had been present during the past few years and because of the progressive loss of weight (Fig. 1) in spite of normal blood chemistry. In the next ten days he felt much

blood pressure, which had previously been 120/80, was 78/50, temperature 97.6°, and respirations 22. Sugar and a trace of acetone were present in the urine, but the blood sugar was only 135 mg. per cent. A tentative diagnosis of Addison's disease was made and the patient was treated with intravenous saline and 10 cc. of adrenal cortical extract (Eucortone) every four hours and later twice daily, with the addition of 7.5 grams of salt daily by mouth. Dr. Lawrence kindly asked me to confirm the diagnosis and collaborate in the treatment of the patient. On March 14, 1942, the serum sodium was 302 mg. chloride 353 mg. and potassium 25 mg. per cent, and the alkali reserve (CO_2) was 67 volumes per cent. Apart from these blood findings and their subsequent return to normality under specific therapy, I felt that the diagnosis of Addison's disease in a diabetic could be made on the following grounds:

1. Progressive improvement in carbohydrate tolerance with recurrent hypoglycemia necessitating drastic reduction in insulin dosage; and instability of blood sugar concentration, together with hypersensitivity to insulin.
2. Weakness, wasting, nausea and vomiting, in spite of good control of the diabetes.
3. Pigmentation—although slight and not on mucous membranes.
4. Hiccups—one of a small group of accessory symptoms, the others being grimaces, curling up under the bedclothes, and conjunctivitis.

Owing to the patient's capricious appetite and his hypersensitivity to insulin, blood sugar control was very difficult in the first few weeks, insulin requirements being reduced to 12 units twice daily, with an occasional smaller dose at midday. Blood sugar on March 16 was 378 mg.; on March 30, 56 mg.; and on April 7, 1,540 mg. per cent, one of the highest values ever recorded. This was at 1 p.m. During the day, after 100 units of insulin intravenously, the blood sugar values were 917 mg. at 4:00 p.m., 495 mg. at 6:00 p.m., 61 mg. at 10:00 p.m., and 45 mg. the next morning.

Owing to the emaciated condition of the patient, the injection of large doses of cortical extract was technically difficult and painful. This was therefore replaced by desoxycorticosterone, 20 mg. injected daily. After a few days both breasts were enlarged, and painful (an occasional result of this therapy) and edema of the face and legs was present. After a very stormy course and several months in hospital, the patient was discharged reasonably well, but weak, with instructions to have injected 30 cc. of cortical extract daily, and 16 units of insulin twice daily.

The blood urea on admission was no less than 235 mg. per cent. Within a few days it fell to 140 mg. per cent, and later to 38 and 26 mg. per cent. There were no casts in the urine. The blood showed a severe secondary anemia with hemoglobin 58 per cent, red cells 2,800,000, leucocytes 9,000; polymorphs 70 per cent, lymphocytes 18.5 per cent, eosinophils 3.5 per cent, and large monocytes 8 per cent. There was a response to iron therapy. Radiography of chest, abdomen and gastro-intestinal tract showed nothing abnormal. A series of chemical studies (Dr. C. H. Gray) of serum electrolytes corresponded with the clinical condition, with occasional anomalies. Blood pressure returned to normal, 120/80.

The patient was a physician, working part-time in a biochemical laboratory and he tended to look after himself. However, he came to see me in June 1943, and stated that his diabetes was controlled by 12 units of soluble insulin twice daily, and that he was also injecting himself with cortical extract (Eschatin), 15 cc. twice daily. His general condition was fair and his blood pressure was 120/80. Serum sodium was 306, chloride 348 and urea 47 mg. per cent, and the alkali reserve (CO_2) was 52 volumes per cent. His wife, a nurse, stated that he became very ill when he tried to reduce the Eschatin to 10 cc. twice daily. In October, 1943, he replaced the Eschatin by desoxycorticosterone acetate, and found that 10 mg. injected daily, or 50 mg. rubbed into the skin daily, kept him in

The infantilism in this case must be attributed to the diabetes mellitus commencing before puberty; nevertheless the patient stated that he was potent in married life until the last few years. There was one daughter. The genitalia were on the small side, and there was appreciable loss of pubic and axillary hair and outer eyebrows. The adrenal insufficiency was treated initially by cortical extract and later by desoxycorticosterone injections and implantation. Although one implantation of 800 mg. of desoxycorticosterone and another of 900 mg. were without untoward effect, a later implantation of 800 mg. produced temporary edema and a still later implantation of 700 mg. of desoxycorticosterone and 400 mg. of testosterone resulted in excessive retention of fluids, which gave way to dehydration on the removal of 300 mg. of desoxycorticosterone and 200 mg. of testosterone and the giving of potassium citrate by mouth. The effect of testosterone on water and salt retention must be considered when this medication is added to desoxycorticosterone therapy. Ultimate death in coma was probably due to adrenal insufficiency and certainly not to diabetic coma.

Although this patient was reasonably well controlled by the administration of insulin and desoxycorticosterone there was a progressive loss of weight and strength (Fig. 1).

Case 3. L. X., a male aged 20, who was serving as an officer in the Royal Navy, was well until the age of 18½, when he began to lose weight and energy. He weighed 140 pounds at the age of 16, and 130 pounds when first seen by me in February 1947. During the preceding six months his appetite had declined further, and he felt the cold more, and in the last two months he had had frequent hiccups. Dr. S. Smith, the family doctor, had noticed some brown pigmentation on the face, and sent him to the writer as a probable case of Addison's disease. On examination he was seen to have pigmentation of the face, neck, periorbital tissue, and nipples, and slight pigmentation of the back of the hands. There was gross pigmentation of the upper gums, and a pigmented line along the lower lip. His pulse was 60, and blood pressure 120/80 at the time of examination, but 100/60 a few weeks later when lying quietly in bed at Willesden General Hospital, where he was admitted for investigation and treatment. On clinical grounds, the diagnosis of Addison's disease was definitely justified.

On February 26, 1947, the serum sodium was 302 mg., the serum potassium 31.4 mg. (very high), and the serum chloride (as NaCl) 440 mg. per cent. The blood urea was only 29 mg. per cent, and the urea clearance test showed 127 per cent of normal the first hour and 160 per cent the second hour. This test repeated a month later gave values of 116 and 110 per cent in the first and second hours. The Kepler test gave a positive result in the first procedure, the night volume of urine being 375 cc., and the largest day volume per hour, 230 cc.; but the Kepler index was 32 and higher than that given for Addison's disease, which is usually well below the upper limit of 30. Most of my cases of Addison's disease have given indices below 15, and this case is unusual in this respect. The high urea clearance test may indicate one factor in this result. The Kepler test was repeated some weeks later, after desoxycorticosterone therapy (which does not appear to change its validity), and still gave an identical index of 32. X-ray examination of the chest showed "healed lesions present in the left subapical region but no evidence of active

later as showing the following values: sodium 321, potassium 24, chloride 398 and urea 57 mg. per cent. On December 5, five days previously, before any untoward signs were obvious the figures were sodium 324, potassium 24, chloride 332, and urea 54 mg. per cent; and alkali reserve (CO_2) 65 volumes per cent. It is difficult to understand why the potassium was not below 20 on December 11, as this is a usual finding with overdosage. However, not waiting for the result of assay, I removed on the afternoon of December 11, 300 mg. of implanted desoxycorticosterone and 200 mg. of testosterone, and during the night prescribed a total of 5 grams of potassium citrate by mouth on the basis of the clinical diagnosis of overdosage with desoxycorticosterone. It seems probable that the sodium-retaining effect of testosterone added to that of desoxycorticosterone was a factor of importance. The next morning the edema was no longer present; in fact the patient looked so dehydrated that intravenous glucose and Eucortone were commenced, but the patient died within twenty-four hours. It was incidentally noted that the eyebrows, pubic and axillary hair were very scanty. Blood sugar estimations on the afternoon of December 12, during intravenous glucose therapy, were 388 and 420 mg. per cent. The urine was sugar free and acetone free but contained albumen and granular casts.

Autopsy

Autopsy showed atrophy of the adrenals and pancreas. Apart from a small heart and a terminal bronchopneumonia, the rest of the findings were negative.

Suprarenals. The right suprarenal could not be identified macroscopically and no adrenal tissue was revealed microscopically. The left suprarenal was small, and enclosed in thick fibrous tissue. The cortical cells remaining showed almost complete absence of lipid in the cytoplasm. There was no evidence of medullary tissue.

Pancreas. This was small, and the islet cells were diminished in numbers and small in size.

Pituitary. The basophil cells appeared to show fewer granules than normal.

Testes. Although of normal size, there was arrest of spermatogenesis at the spermatogonia stage, and some diminution in the number of interstitial cells.

Thyroid. This gland was rather small and showed some lymphoid infiltration.

Comment: case 2

This is a very exceptional case inasmuch as diabetes mellitus commenced at the age of 11, and Addison's disease did not develop until twenty-one years later at the age of 32. The treatment of both conditions was successfully maintained for a further five years and when the patient died at the age of 37, atrophy of the adrenals and of the pancreatic islet cells was found. The long period between the first appearances of the two diseases appears to preclude a common etiology. Some months before Addison's disease was obvious clinically, repeated hypoglycemic attacks necessitated progressive reduction of insulin dosage from 72 units to 48 units daily, and ultimately the daily insulin requirement was only 6 units. The patient showed the usual instability of blood sugar values when both adrenal and islet cell insufficiency were present at the same time. Some extremely high values for blood sugar were obtained, *e.g.*, 917 and 1,540 mg. per cent; and, in contrast, hypoglycemic values of 56 and 45 mg. per cent. Acetone was present at times in the urine.

On March 5, daily injections of 5 mg. desoxycorticosterone acetate were started and after a week the serum sodium was 339 mg., the serum potassium 15 mg., and the serum chloride (NaCl) 577 mg. per cent. These figures might indicate slight overdosage but the patient was very much better, gaining weight and strength and showing no edema. On March 18, therefore, I implanted 400 mg. of desoxycorticosterone. The injections of this material were stopped on March 22. The patient left the hospital on April 14, 1947, feeling very well and having gained 9 pounds in weight. The blood pressure was 120/70. There was no edema and there were no crepitations at the base of the lungs. Pigmentation had decreased.

CASE 3-AGE 20

SUGAR TOLERANCE CURVE.

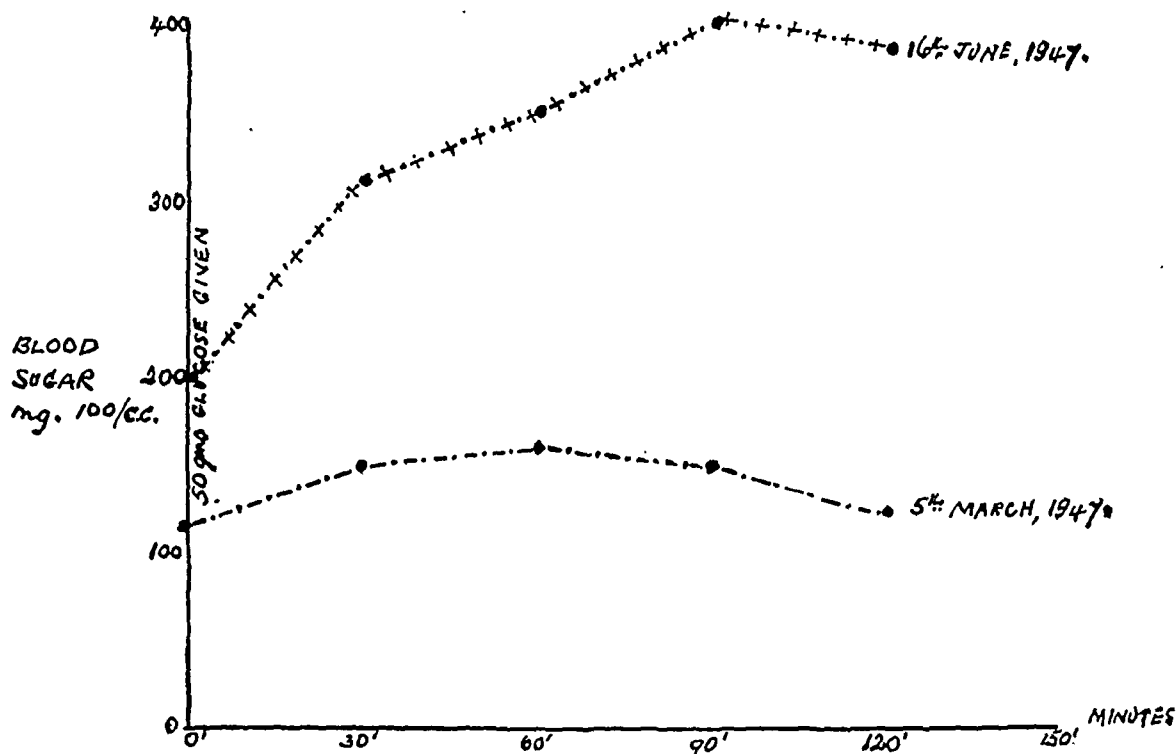


FIG. 3.

The patient continued well, except for some cramps in the arms and legs, until June 1947, when he complained of weakness, lassitude, thirst, and polyuria, and some swelling of the left parotid gland which was painful on chewing and prevented complete opening of the mouth. He had not been in contact with mumps. The thirst and lassitude appears to have preceded the swelling of the left parotid gland by some weeks. He was again admitted to the Willesden General Hospital on June 13, 1947. The urine contained sugar and acetone, both in considerable quantities. The blood sugar (fasting) was 320 mg. per cent. The blood pressure was 90/60. The swelling of the parotid gland subsided after a few days, as did a slight pyrexia, 99° F.

A carbohydrate tolerance test under the same conditions as the previous one in February 1947 gave a typical diabetic curve; fasting, 200 mg.; 30 minutes, 310 mg.; 60 minutes, 350 mg.; 90 minutes, 400 mg.; and 120 minutes, 380 mg., per cent (Fig. 3). The urine contained much sugar (red with Fehling's solution), much acetone and in the

tuberculosis"—(D. Gordon). In this connection it is of interest to note that the patient's father died of pulmonary tuberculosis at the age of 37, and the father's sister died of abdominal tuberculosis as a child of 3. No calcification was detected radiographically in the suprarenal area, but the presumptive evidence was in favor of diagnosing a tuberculous lesion of the adrenals. Sedimentation rate was normal; 3 mm. in the first hour, and 7 mm. after two hours.

CASE 3 - AGE 20
INSULIN SENSITIVITY TEST.

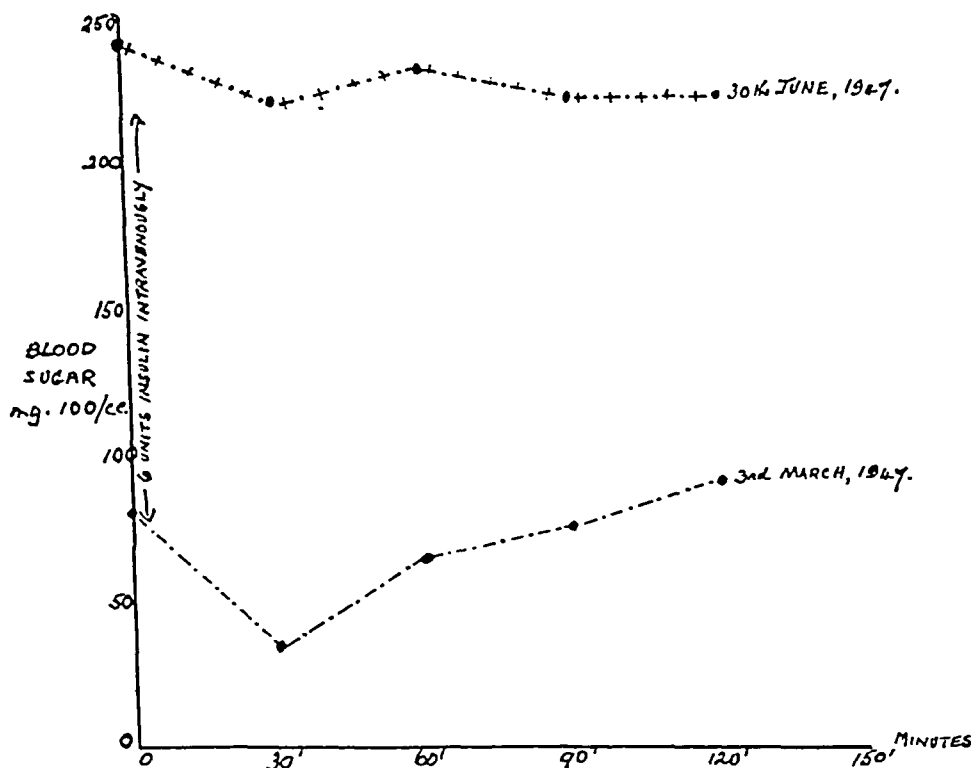


FIG. 2.

The urine was normal on routine testing. Fortunately for later developments, I was interested in carbohydrate metabolism in Addison's disease, and on February 21, 1947, a carbohydrate tolerance test was carried out and gave the following blood sugar values: fasting 115 mg.; after 50 Gm. glucose—30 minutes, 150 mg.; 60 minutes, 160 mg.; 90 minutes, 150 mg.; 120 minutes, 125 mg.; 150 minutes, 110 mg. per cent. There was a slight reduction of Fehling's solution in the urine collected after 30 minutes but not in the other specimens. This tolerance curve is not a diabetic one but values during fasting are slightly above normal and after two hours the blood sugar value is still 125 mg. per cent. However, the blood sugar (fasting) on March 3, 1947, was only 90 mg. and the insulin sensitivity test was normal (Fig. 2). The importance of these observations will be more obvious later.

could be felt about two inches below the site of incision, and were very small. The blood pressure was 105/70. On January 16, 1948, serum sodium was 306 mg., potassium 24.5 mg. and chloride (NaCl) 676 mg. per cent (H. Scott-Wilson). The chloride was rather high, considering the low sodium and high potassium. On January 21 I implanted 300 mg. of desoxycorticosterone, and the administration of salt by mouth was stopped. On January 27 he was ill and complained of nausea. He also vomited and then refused food. He looked dehydrated. Clinically, since the diabetes was controlled and there was no evidence of edema, adrenal insufficiency was diagnosed, although the blood pressure was 110/70. He was given 10 cc. of cortical extract (Eucortone) intramuscularly, and this was repeated in four hours. He felt much better the next morning and continued well. Nausea disappeared and appetite returned. However, blood taken at the time of malaise gave little indication of the clinical diagnosis of adrenal insufficiency: sodium 309, potassium 15, and chloride 705 mg. per cent. The response to Eucortone seems to me to have justified the clinical view. The patient left the hospital on February 7, 1948, feeling very well and has so remained.

Comment: case 3

This is an undoubted case of diabetes mellitus following upon Addison's disease. It is of interest in that the lesion of the adrenals was almost certainly tuberculous, in view of the family history. It is questionable whether the parotitis was mumps, and whether it precipitated or initiated the diabetes mellitus. Mumps may be complicated by pancreatitis and transient glycosuria (Wilder, 10), but it is not established as a causative agent in diabetes mellitus. In this case it appears more probable that it aggravated a pre-existing diabetes since the blood sugar values were very high, and ketosis was present at the time of onset of the swelling of the parotid gland (only the left side was involved). It is of interest, since adrenalectomy is said to minimize ketosis, that when the diabetes was uncontrolled, ketones and acetone were present in the urine. This patient, however, could not be regarded as completely adrenalectomized.

The first procedure of the Kepler water test was positive twice before diabetes developed, and negative after diabetes developed. The index was negative throughout and grossly negative when the diabetes was severe and uncontrolled. It would appear that the Kepler index is no indication of adrenal function in the presence of diabetes mellitus.

As regards carbohydrate tolerance, the test in March 1947 suggested possibly a mild diabetes mellitus, but the test in July 1947, a very gross diabetes. The insulin sensitivity test was normal in March, but distinctly different in July, when the blood sugar showed little response. The diabetes was easily controlled by insulin, although blood sugar values were unstable and hypoglycemic attacks indicated clinically a progressive improvement in tolerance or a progressive sensitivity to insulin.

The adrenal insufficiency was controlled by desoxycorticosterone, first by injection and later by implantation, but the observed values for blood electrolytes did not always correspond to the clinical conditions.

60-minute specimen, some aceto-acetic acid. The insulin sensitivity curve was distinctly different from that of February, and indicated only a slight action of 6 units of insulin intravenously. Blood cholesterol was 130 mg. per cent. Serum sodium was 300 mg., potassium 17.9 mg., and chloride (as NaCl) 544 mg. per cent. Blood count: red cells, 4,030,000; hemoglobin 78 per cent; leucocytes 4,800; polymorphs 47, lymphocytes 49, monocytes 2, basophils 1, and eosinophils 1 per cent. A Kepler test gave an unexpected index of no less than 208, largely due to the fact that with a night volume of 425 cc. of urine, the day volumes per hour were 120, 550, 210 and 95 cc. The 17-ketosteroids (Dr. W. Payne) were 2.5 mg. per liter, and 6.4 mg. per day.

On July 3, a three-day nitrogen balance test was carried out by Dr. G. Discomb. The daily intake of nitrogen was 60 Gm. and the results were as follows:

	Urine				
	Volume cc.	Sugar Gm. %	Nitrogen Gm. %	Total sugar Gm.	Total nitrogen Gm.
1st day	2,400	2.5	0.59	60.0	14.1
2nd day	1,630	2.9	0.71	47.3	11.5
3rd day	2,930	2.6	0.55	76.2	15.2

Expected nitrogen excretion = total protein intake/6.25 = 9.58 Gm.

Mean observed nitrogen excretion = 13.6 Gm.

During the above test the diabetes was partially controlled by soluble insulin, 18 units in the morning and 16 units in the evening, and 180 Gm. of carbohydrate daily. Subsequently it was necessary to raise the dose of insulin to 28 units in the morning and 24 units in the evening, but later this was reduced to 16 units in the morning and 12 units in the evening because of hypoglycemic attacks. The patient left the hospital on July 28, 1947, with the diabetes well controlled. He felt very well and had regained a few pounds in weight lost prior to insulin therapy. His blood pressure was 100/70, and in view of the implantation of desoxycorticosterone in April, no further treatment for the adrenal insufficiency was prescribed.

He continued to remain well even in December 1947, but at that time it was felt advisable to add 1 gram of salt by mouth three times daily to the therapy, since the blood pressure was only 90/60. On January 7, 1948, he was again admitted to Willesden General Hospital following a telephone call from his mother that he had become semi-conscious several times during the last few days. He arrived thus in the hospital, the urine containing no sugar or acetone, and the blood sugar being 65 mg. per cent. The attacks were diagnosed as being due to hypoglycemia and they responded to glucose by mouth, an increase of daily carbohydrate intake from 180 to 220 Gm., and reduction of insulin to 14 units in the morning and 12 units in the evening. However, the blood sugar was somewhat unstable, values of 143, 248 and 320 mg. per cent being obtained at different times, and it was necessary subsequently to raise the insulin to 18 units and 16 units respectively.

The patient's general appearance at this time was good, but the pigmentation of the face and mucous membranes had increased. The testes and penis appeared normal but the pubic hair was somewhat scanty. The implanted desoxycorticosterone pellets

TABLE 1

Author	Sex	Age yrs.	Duration of diabetes before death, yrs.	Duration of Addison's disease before death, yrs.	Units of insulin required before Addison's disease	Units of insulin required after Addison's disease	Autopsy		
							Adrenals	Pancreas	Other organs— comments
1. Ogle, J. W., 1866 (11)	Male	55	<1	No clinical evidence in life	0	0	Tuberculosis.	—	Tuberculosis of lungs.
2. West, S., 1890 (18)	Male	49	<1	<1	0	0	Fibrosis.	?	Liver ++. Hemochromatosis.
3. Rabé, M., 1900 (13)	Male	49	3	?3	0	0	Tuberculosis (no pigment deposited).	Fibrosis. Hemosiderin deposited.	Liver ++. Hemochromatosis.
4. Montgomery, C. A., 1912 (12)	Male	45	1½	No clinical evidence in life.	0	0	Right: caseous tuberculosis. Left: hypertrophy. No tuberculosis.	Some increase in connective tissue; islets not abnormal.	Lungs normal. No pigmentation of skin.
5. Unverricht, 1926 (14)	Male	32	6	<1	40	10	Tuberculosis.	Not mentioned.	—
6. Arnett, J. H., 1927 (37)	Female	39	2	1½	80	20	Atrophy and fibrosis.	Slight fibrosis; islets small and poorly staining.	Acetone and diacetic acid in urine.
7. Rowntree, L. G., and Snell, A. M., 1931, Case 1 (22)	Male	35	2	1	30	?	Atrophy.	Atrophy.	Thyroidectomy but not clinically thyrotoxic.
8. Rowntree, L. G., and Snell, A. M., 1931, Case 2 (22)	Male	?	6	1	?	?	Atrophy.	Atrophy.	Footnote only. No details.
9. Gowen, W. M., 1932 (38)	Female	54	2	2	?	?	Atrophy.	Some increase in connective tissue; hyalinized islets; lymphocytic infiltration.	Not fully documented.

DISCUSSION

Pathology and Etiology

In Table I are given some essential details of 15 cases of adrenal and islet-cell insufficiency that I have been able to trace in the literature, and of 2 cases under my own observation. Of these 15 cases, 2 (Ogle (11) and Montgomery (12)) had no evidence of Addison's disease in life. In Ogle's case the patient had pulmonary tuberculosis and "both suprarenal capsules were finely mottled with an appearance like that of the liver, and each contained a small patch of fibrinous material in the centre." In Montgomery's case the right adrenal was destroyed by caseous tuberculosis, but the left adrenal was untouched by the disease and had undergone compensatory hypertrophy. Three other cases (Rabé (13), Unverricht (14), and Bowen and associates (15) (16)), had tuberculous adrenals, so that a tuberculous lesion of the adrenal gland was present in 5 of the 17 cases. In the remaining 12, the lesion was atrophy or fibrosis of the adrenal glands.

The terms atrophy and fibrosis are used somewhat arbitrarily by different recorders, and both terms indicate the same lesion, the histologic picture of which bears a closer resemblance to a toxic necrosis (*cf.* the liver) than to a simple atrophy. There may, however, be a good deal of fibrous tissue in or around the adrenal gland and not infrequently it is almost impossible to detect any adrenal tissue macroscopically, so that the tissue in the anatomic neighborhood must be taken for section. In Nix's case (17) only one atrophied adrenal remnant could be found and that only on microscopy of regional tissue. Microscopically the cortical layer or a remnant of it is seen to be very thin, and almost entirely replaced by distended capillaries lying in a sparse, delicate, connective tissue, infiltrated with lymphocytes, plasma cells and histiocytes. Small areas of surviving and regenerating cortical cells may be detected. The medulla is usually of normal size and pattern except for some lymphocytic infiltration. In West's case (18) the adrenal lesion is correctly termed fibrosis, but there is little doubt that hemochromatosis was also present, affecting the liver. West recorded: "the notes of the autopsy have unfortunately been mislaid but so far as I remember there was nothing of importance except the condition of the suprarenal capsules. These were embedded in dense fibrous tissue, and sections showed no trace of suprarenal structure." When the lesion is tuberculous, the greater part of the adrenal gland is usually destroyed by caseous tuberculosis.

The lesion of the pancreas is also described as fibrosis or atrophy, but in the majority of cases the degree of fibrosis is slight and consists only of some increase in connective tissue. The islets of Langerhans are decreased in number, small in size, and stain poorly, and they may be hyalinized.

There is often some lymphocytic infiltration.

Of the 11 uncomplicated cases of atrophy of adrenals and pancreas (Nos. 6, 7, 8, 9, 10, 11, 13, 14, 15, 16 and 17) in 6 of these (Nos. 6, 7, 9, 13, 14 and 16) the adrenal and islet-cell insufficiency were manifest clinically within the same year (twelve months) if not simultaneously. It is therefore my opinion (first stated Simpson (1)) that the lesion of atrophy (or fibrosis) in the adrenal gland and that of the islet cells of the pancreas have the same origin and that it is the result of some infection, probably virus in character. It is, of course, known that mumps may affect the pancreas (including the islet cells) and the testes. The probable importance of other virus infections, *e.g.* influenza, and specific fevers, in the etiology of endocrinopathies is not so well recognized, but has been pointed out by Simpson (19). The fact that clinical manifestations of Addison's disease and diabetes mellitus do not coincide in some patients does not exclude a simultaneous pathologic lesion since 1) an infective lesion may be progressive in its effects; 2) clinical insufficiency is not manifest until the greater part of an endocrine gland is destroyed, and 3) a tendency to diabetes from islet-cell insufficiency is counterbalanced by the ameliorating effect of adrenal cortex insufficiency. Thus in Thorn and Clinton's patient (20), still alive, the clinical diabetes did not develop until four years after the Addison's disease. However, a carbohydrate tolerance curve three years before the onset of the clinical diabetes, and another two years before, were diabetic in type, the later curve showing a raised blood sugar concentration as well as a slow return to normality. In my third case (patient still alive) indications of latent diabetes, as judged by the tolerance curve, were present some months prior to the onset of clinical diabetes. In my first case (number 10), although diabetes was not manifest until a few weeks before death, a year before this it was noticed that the patient drank large quantities of water and passed a great deal of urine for some weeks. It is also difficult in this case to know whether the stoppage of growth at the age of 13 and the persistent infantilism was due to the latent Addison's disease or the latent diabetes, from both of which the patient died at the age of 16. Of the remaining 4 cases (Nos. 8, 11, 15, 17) in which uncomplicated atrophy of the adrenals and of the pancreas was the lesion, that of Rhind and Wilson (21) is comparable to my first case, in that the Addison's disease appeared to precede the diabetes by some two years; in the case of Rowntree and Snell (22) and that of Devitt and Murphy (23), the diabetes preceded the Addison's disease by five and three years respectively; whereas in the case of Simpson (present paper; case 2) the diabetes preceded the Addison's disease by no less than twenty-one years. In this last case, and possibly in the 2 former ones, it would probably be conceded that the occurrence of the two diseases in one patient was a coincidence.

TABLE 1—(continued)

Author	Sex	Age yrs.	Duration of diabetes before death, yrs.	Duration of Addison's disease before death, yrs.	Units of insulin required before Addison's disease	Units of insulin required after Addison's disease	Autopsy		
							Adrenals	Pancreas	Other organs— comments
10. Simpson, S. L., 1932 (1)	Male	16	<1	1	0	15	Atrophy; fibrosis.	Fibrosis; small islets; lymphocytic infiltration.	Acetone but no diacetic acid in urine.
11. Rhind, E. G. G., and Wilson, A., 1941 (21)	Female	32	<1	2	0	48	Atrophy; fibrosis.	Fibrosis.	Hypertrophy of thyroid.
12. Bowen, B. D., Koepf, G. F., Bissell, G., and Hull, D., 1942 (15)	Not given	Not given	Not given	7 but after diabetes	Not given	Not given	Tuberculosis.	"A small pancreas."	Description very brief.
13. Koepf, G. F., and Bowen, B. D., 1943 (30)	Female	54	2	2		<5	Atrophy.		Unpublished; brief quotation by Thorn and Clinton, 1943.
14. Nix, N. W., 1943 (17)	Male	39	<1	<1	40	7	Atrophy.	No obvious abnormality.	Acetone (trace) in urine.
15. Davitt, J. S., and Murphy, F. D., 1947 (23)	Female	28	4	1	30	10	Fibrosis.	Fibrosis; decrease in number of islet cells.	Familial diabetes.
16. Bernstein, D. E., 1948 (31)	Male	44	0	8½	50	4	Fibrosis.	Fibrosis; small islet cells with few beta cells.	Fibrous thyroid. Acetone and diacetic acid in urine.
17. Simpson, S. L., 1948 (present paper)	Male	37	20	5	72	0	Atrophy.	Small pancreas; islets small and diminished in number.	Acetone and aceto-diacetic acid in urine.

The specific question as to whether hemochromatosis can cause clinical Addison's disease by deposition of hemosiderin in the adrenal cortex (and in the absence of any other adrenal lesion) is not easy to answer with certainty, although it would appear to be in the negative. Thus in a statistical study of the pathology of 566 cases of Addison's disease, Gutman (25) does not mention hemochromatosis as the pathologic lesion. On the other hand Hellier (26) found that in 57 cases of hemochromatosis, hemosiderin was present in the skin in 48 and melanin in 35. In only 8 instances was hemosiderin present without melanin. Pigmentation of the mucous membranes occurred in 11 per cent of cases of hemochromatosis. If an increased deposit of melanin is taken as an indication of adrenal insufficiency, the only other manifestation of this in hemochromatosis is the not uncommon occurrence of hypotension (Sheldon (27)) but the latter might also be due to destruction of the anterior pituitary gland by hemosiderin. Although in no case in which melanin was present in excess in the skin, was iron absent from the suprarenals, iron was sometimes present without an increase of melanin. Further, melanin deposition in the adrenal gland is usually limited to the zona glomerulosa, and even there many cells escape. The increased deposition of melanin in the skin may nevertheless be due to irritation from hemosiderin deposition. Although the clinical evidence for adrenal insufficiency in hemochromatosis is not strong, and although it seems clear that the full clinical picture of Addison's disease does not result from hemosiderin deposition in the adrenals, yet some disturbance of adrenal function through hemosiderin deposition must be regarded as probable, especially as such an effect on function does result in other endocrine glands, *e.g.* pancreas, pituitary, and testis.

I have not included in this series the case of Brookfield and Corbett (28) since the adrenal lesion was very unusual, but it should be briefly mentioned. The patient was a woman of 61, who had had diabetes for six months, and was admitted to the hospital in severe diabetic coma with ketonuria and a blood sugar of 1,040 mg. per cent. (The authors mention that the highest blood sugar recorded by Joslin (29) was 1,490 mg. per cent which is less than the 1,540 mg. per cent found in my case 2.) The patient had pyrexia and leucocytosis and was refractory to insulin for some days, but then responded to large doses, *e.g.* 160 units in 24 hours, a blood sugar of 75 mg. being attained. Death occurred fourteen days later. At autopsy pancreatic islet cells showed hyaline and fibrotic changes, and both adrenals were cystic, like semicollapsed balloons. The thin shell enclosing the fluid consisted of atrophic cortical tissue, with considerable atrophy of the zona fasciculata. All organs showed congestion and fibrosis and the right kidney, multiple miliary abscesses. The authors postulated death from adrenal insufficiency although there was no evidence of Addison's disease in life.

When the adrenal lesion is tuberculous and the pancreatic lesion atrophic, one would be justified in postulating that the two lesions were definitely separate and coincidental. However, it is perhaps necessary to remember that apart from generalized miliary tuberculosis, the pancreas is not involved in a blatant tuberculous lesion. Whether or not the tuberculous toxin can produce a fibrosis or atrophy of the islet cells of the pancreas in the absence of any local indication in the pancreas of its tuberculous origin is, of course, highly speculative.

In the metabolic disorder of hemochromatosis, hemosiderin can be deposited in the liver, spleen, pancreas, adrenals, anterior pituitary, testes, and other organs. It therefore might be expected that Addison's disease and diabetes mellitus could occur together in a patient suffering from hemochromatosis. In the present series (Table 1) there are 2 such cases, those of West (18) and Rabé (13). Unfortunately, West's is very poorly documented pathologically, the only evidence being fibrosis of the suprarenals. However, clinically the patient had definite diabetes mellitus with 56 pounds loss in weight, the liver was grossly enlarged, and the skin was of "greying cyanotic hue much like that observed in chronic silver staining." Addison's disease was thought of before death, but the clinical evidence was not strong. In Rabé's case (13) diabetes was definite, "the grey discolouration of the skin immediately made one think of Addison's disease or bronzed diabetes," the liver and spleen were grossly enlarged, and the patient died in coma. The evidence of Addison's disease in life was slender, but both adrenals were completely destroyed by caseous tuberculosis without deposition of hemosiderin. The liver showed typical hypertrophic cirrhosis with pigmentation and the pancreas "of sclerotic consistency, presented as the liver, and with a dark chocolate colour." At the apex of the right lung there was "a small nodule surrounded by fibrosis that gave the slightest suggestion of tubercle." The adrenals were "much increased in size, transformed entirely into a block of cartilaginous consistency, which recalled, after section, the aspect of tuberculous matter in a state of crudity; it was in effect a whitish amorphous substance with no pigmentation; it infiltrated completely the organ and made impossible any distinction between cortex and medulla." This case, therefore, must be considered as a coincidence of hemochromatosis affecting the pancreas and caseous tuberculosis affecting the adrenals. Achard and Leblanc (24) described a case in which the morbid anatomic findings were caseous tuberculosis of the adrenals and hemochromatosis of the liver, but there is no mention of the pancreas and no evidence of diabetes in life. There was no good clinical evidence of Addison's disease in life except pigmentation of the skin and mucous membranes, but the pathologic findings are of interest.

were present, though in slightly diminished numbers." Apart from infantilism the scantiness of pubic and axillary hair met with in case 2, and to a much less extent in case 3, has also been observed by me in women with Addison's disease of some years' duration, and is probably a reflection of the low androgen secretion of the diseased adrenal cortex, as well as of hypogonadism in the case of the male.

Cases not coming to autopsy

Although this paper, including its clinical and pathological conclusions, is based on 17 cases coming to autopsy, there should be added to these for completion 6 cases without autopsy. Armstrong (33) recorded a case of a man of 35 with diabetes of acute onset, controlled by 56 units of insulin, who, within a few months of initial treatment, found it necessary to reduce progressively the dose of insulin, and within a year developed a craving for salt, severe hypoglycemic reactions from 6 units of insulin daily, and pigmentation of the skin. During the latter part of a period of five years' observation, "the most striking change has been a progressive hypothyroidism." McCullagh's case (30), a pale thin young man (? age) who had diabetes for seven years with daily insulin requirements varying between 40 and 120 units, began to complain of weakness, abdominal pains, anorexia and a loss of 30 pounds in six months. Abdominal laparotomy showed nothing abnormal but the repeated postoperative response to intravenous saline led to the diagnosis of Addison's disease. The Kepler index was positive, 6.9. Thorn and Clinton's case (20) was a man of 23 years, who developed typical Addison's disease, and who during three years of treatment with desoxycorticosterone implantations had attacks of negativism and catatonia due to hypoglycemia. He then manifested diabetes clinically and biochemically, with persistent ketonuria. His daily insulin requirements varied between 10 and 18 units. His carbohydrate tolerance curve was of the diabetic type at least two years before the onset of clinical diabetes. In the early days of treatment, the commencement of insulin therapy appeared to aggravate the tendency to low serum potassium levels, with accompanying lower limb paralysis. Withdrawal of insulin produced a striking increase in potassium excretion. The administration of a single dose of Kendall's compound E resulted in a striking decrease in glucose tolerance, a marked increase in the excretion of glucose, nitrogen, phosphorus, sodium and chloride, but not of potassium, and a decrease in the respiratory quotient.

Rogoff's case (34) of Addison's disease following adrenal denervation for diabetes mellitus in a male is not well documented but should be mentioned for completeness.

Lowrie and colleagues (35) reported this year the case of a colored

Other endocrine glands

In Rowntree and Snell's first case (22) tachycardia was a feature, and the basal metabolic rate was plus 24 per cent. Although there is no record of the thyroid gland being enlarged, thyroidectomy was carried out, and the patient died next day in adrenal insufficiency and hypoglycemia, with a blood sugar of 50 mg. per cent. The histology of the gland showed parenchymatous hypertrophy and the case has since been repeatedly referred to in the literature as illustrating the coexistence of thyrotoxicosis together with Addison's disease and diabetes mellitus. The evidence hardly justifies the title, and it is of interest to note that in Rhind and Wilson's case (21), without any clinical evidence of thyrotoxicosis, the thyroid gland showed hyperplasia. In the other autopsy cases, where recorded, the thyroid gland was normal but with some lymphocytic infiltration. McCullagh (30) reported the coexistence of myxedema with diabetes in 5 cases; Bernstein's (31) recent autopsy report on a case previously described clinically by Bloomfield (32) illustrates myxedema and Addison's disease superimposed on diabetes mellitus, with atrophy of the thyroid, adrenals and pancreas at autopsy.

Rhind and Wilson (21) mention that "attention has been focused on the relationship between the anterior lobe of the pituitary and other endocrine glands." It is well known that the basophil cells are decreased in number and perhaps size in Addison's disease, and in Rhind and Wilson's case there was some degeneration of all three types of pituitary cells. Such changes in the pituitary gland are probably secondary, or incidental, to the adrenal and pancreatic lesions. The atrophy or cirrhosis of the adrenal glands found in the above series of cases is a distinctly different pathologic lesion from the hypoplasia or involution of the adrenal gland that follows hypophysectomy or that occurs in Simmonds' disease: nor of course are the pancreatic islet-cell changes comparable. Clinically diabetes does not occur in Simmonds' disease or panhypopituitarism. In other words, there are no grounds for postulating that the syndrome of diabetes mellitus with Addison's disease, when associated with atrophy of the adrenals and the pancreas, can be initially due to changes in the pituitary gland. Rhind and Wilson (21) point out that "the pathological changes suggest that there is no relationship between the number of basophil cells in the anterior lobe of the pituitary and the blood sugar level."

In my 1932 (1) case infantilism was present, and the testis showed "semiferous tubules separated by much interstitial tissue in which no interstitial cells could be recognized. Few had a lumen and some of them contained spermatocytes." In my second case, impotence was present for some years, and in the testis "spermatogonia were present but spermatogenesis appeared to be arrested at a late stage. Interstitial cells of Leydig

hyperglycemia was initially refractory to insulin, although autopsy showed cystic atrophic adrenal glands. The severity of the diabetes is aggravated by cortical extract or by members of the corticosterone group, *e.g.* Kendall's compound E. It is not thus influenced by desoxycorticosterone.

There appears to be a progressive loss of weight and fat in these patients, even when substitution therapy is approximately adequate as judged by biochemical data. The combined therapy is cumbersome and not easy to carry out although the desoxycorticosterone implantation method for Addison's disease limits the injections to insulin only, and is therefore in my view the method of choice. The tendency to ketosis appears to be diminished (as follows adrenalectomy in phloridzin diabetes), although both acetone and aceto-acetic acid may be present at times with glycosuria and hyperglycemia. Thorn and Clinton (20) suggest that ketosis is present only when dehydration is severe. This does not appear to be consistently the case in the present series, although it may well be that dehydration from adrenal insufficiency renders the patient more sensitive to ketosis and that this may be associated with anorexia and the poor intake of food. It is certainly true that death does not occur from diabetic coma, but from adrenal insufficiency or possibly from hypoglycemic coma. It is perhaps of interest to recall the case, described by Anderson (36) of a man of 33 admitted to the hospital in hypoglycemic coma without previous history, and with a blood sugar of 40 mg. per cent. He was found at autopsy to have a large left adrenal carcinomatous tumor weighing 400 grams, and a small fibrous right adrenal gland.

When testosterone is implanted, together with the desoxycorticosterone, for the treatment of an associated impotence or for its anabolic nitrogen-retaining effect, it should be remembered that it also produces a retention of sodium, so that the dose of desoxycorticosterone implanted should be lowered to allow for this. It may, however, also result in decreased potassium excretion.

Of the 3 patients under my care recorded in this paper, the first showed infantilism and the second partial infantilism. In the latter case, the diabetes commencing at the age of 11 and inadequate treatment initially, may have been the cause. In the former, it would appear that adrenal insufficiency was responsible for the infantilism. Either diabetes mellitus or Addison's disease commencing before or at puberty may be associated with infantilism, especially when adequate substitution therapy is not consistently maintained.

SUMMARY

1. Three cases of Addison's disease and diabetes mellitus are described fully with autopsy findings in the two patients who died.

female, aged 30, who was first seen in December 1944, with emaciation, nausea and diarrhea, increased pigmentation of the skin, and new patches of pigment on the gums. The diagnosis of Addison's disease was confirmed by biochemical data, and the condition responded to desoxycorticosterone implantation. Six months later thirst and polyuria led to the discovery of sugar and acetone in the urine, and a blood sugar of 346 mg. per cent. However, the administration of 5 units of soluble insulin daily led to hypoglycemia with attacks of disorientation and even coma, which were obviated by substituting globin insulin.

My third patient (described above) remains alive and well, although in a recent temporary phase of acute adrenal insufficiency, he had a persistent hypoglycemia which nearly proved fatal.

Comment

We have seen from the previous section on pathology and etiology that clinical manifestations of adrenal and islet-cell insufficiency are not infrequently simultaneous, but that either may precede the other by some months, or occasionally years. Carbohydrate tolerance curves, when carried out, have indicated a disturbance of islet-cell function months or years before clinical diabetes manifested itself (*e.g.* Simpson's case 3; and Thorn and Clinton's case). It is perhaps more unusual for the clinical syndrome of Addison's disease to be present for some months or years before the diabetes, but this was so in 5 cases, Simpson (1), Rhind and Wilson (21), Thorn and Clinton (20), Lowrie and colleagues (35), and Simpson (case 3, present paper). In another of my cases (case 2) diabetes preceded Addison's disease by no less than twenty-one years and the two diseases coexisted for a further five years.

When adrenal insufficiency is superimposed upon a pre-existing diabetes, there is a progressive improvement in carbohydrate tolerance and a striking decrease in insulin requirements. There is also a considerable instability of blood sugar levels as if the mechanism for blood sugar regulation were missing, or impaired, and this is in keeping with the experimental evidence that the adrenal cortex, via the pituitary adrenocorticotrophic hormone, plays an important part in such regulation. In my case 2, blood sugar values varied between 1,540 and 45 mg. per cent, and wide fluctuations were met with on the same day with comparatively small doses of insulin. There is usually a very considerable hypersensitivity to insulin and hypoglycemic attacks are by no means infrequent. They tend to become more frequent as the adrenal insufficiency becomes progressively worse. Nevertheless, if the diabetes is severe, insulin sensitivity with small intravenous doses of insulin may not be manifest at the time of testing (my case 3). In the case of Brookfield and Corbett (28) (*see* pathology section) severe

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2. An additional 15 recorded autopsies are summarized, making 17 in all. The islet cells of the pancreas were reduced in size and staining power, sometimes with hyalinization, and there was an increase in connective tissue and lymphocytic infiltration. The adrenal lesion was atrophy in 10 cases and tuberculosis in 3.

3. Included in the above series are two instances of hemochromatosis, in one of which the adrenal lesion was fibrosis and in the other, coincidental tuberculosis.

4. Clinically, adrenal and islet-cell insufficiency may be manifest simultaneously, or either may precede the other. When Addison's disease comes first, diabetes mellitus may be latent for months or years before it manifests itself clinically or conclusively.

5. The evidence tends to confirm the author's suggestion, first made in 1932, that the coexistence of adrenal and islet-cell atrophy may be due to a common infective lesion.

6. When adrenal insufficiency is superimposed on diabetes mellitus, there is a progressive reduction in insulin requirements, an increase in insulin sensitivity, an instability of blood sugar levels and a proneness to sudden hypoglycemia.

7. The existence, or superimposition, of adrenal insufficiency leads to a progressive loss of weight and of fat, even with reasonable substitution therapy, and ultimately death is usually due to adrenal insufficiency rather than diabetic coma.

8. The Kepler test for adrenal insufficiency is not necessarily valid in the presence of islet-cell insufficiency.

9. Insulin may accentuate the depression of the serum potassium level caused by the administration of desoxycorticosterone and salt; and inadequately controlled diabetes may have the opposite effect. Testosterone may cause a retention of potassium.

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THE EXCRETION OF 17-KETOSTEROIDS. I. NORMAL VALUES IN RELATION TO AGE AND SEX*

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THE purpose of the present study has been to ascertain the range and average rates of excretion of 17-ketosteroids in the urine of normal subjects at various ages as determined by the rapid method (1) previously described. Such data should prove useful for appraising results obtained in the study of endocrinopathies by the now rather large group of investigators throughout the country who have manifested an interest in, and are using the "short" method (1).

MATERIALS AND PROCEDURE

More than 450 twenty-four hour specimens of urine from 120 individuals. 93 male and 27 female, were examined. In the majority of cases three, and often many more, determinations per individual were made. More than half of the subjects were without clinical evidence of any disease. A smaller proportion were considered "normal" although they had one of a variety of disorders of a nonendocrine nature, such as pulmonary emphysema, mild anemia, coronary sclerosis, or psychoneurosis. Five cases of well-controlled diabetes mellitus were also included as we have failed to find any significant variation from normal in the excretion of 17-ketosteroids in such subjects.

Exclusive of 15 children less than 13 years old, the subjects included 85 males ranging in age from 13 to 75 years and 20 females ranging in age from 17 to 64 years. To facilitate interpretation, several arbitrary divisions according to age were made. The children were thus segregated into two groups regardless of sex, including respectively those under 5 years of age and those between 5 and 12 years old. There were 2 girls in the former and 5 in the latter group. The men were divided into four large sections according to age in years: 13 to 16, 17 to 34, 35 to 49 and 50 to 75, with 3, 53, 11 and 18 subjects respectively in each group. The second section was further subdivided into 4 age levels as follows: 17 to 19, 20 to 24, 25 to 29, and 30 to 34 years, in which there were respectively 26, 14, 10 and 3 individuals. Significant variations in relation to age were not noted in

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24-hour specimens were studied. Individual determinations varied from 1.0 to 8.2 mg. per day, and individual averages ranged from 2.4 to 5.3 mg. with a tendency for the older children to show the higher figures. The mean excretion for the group was 3.8 mg. daily. As compared with the younger boys, those between 13 and 16 years of age showed significantly increased values for urinary 17-ketosteroids (average, 9.4 daily) and a wide fluctuation for individual values (4 to 21 mg. daily). The averages for the individual subjects varied from 5.6 to 14 mg. per day.

As compared to other groups, men in the most active period of sexual life, 17 to 34 years, had the highest average daily output of 17-ketosteroids, *viz.* 18 mg., and the widest range of values. Averages for the individual subjects in this group varied from 11 to 27 mg. per day. Figures for individual determinations lay between 9 and 30 mg. per day. Apparently in this period of life, a direct relationship exists between age and the amount of 17-ketosteroids excreted, as the means for the four sub-groups, arranged in ascending order of age, were 17.4, 17.8, 19.4 and 20.7 mg. per day respectively.

Only a moderate decrease in the excretion of 17-ketosteroids was observed in males of 35 to 49 years of age, as compared with younger men. The average for the group was 15 mg. with a range for the averaged individual values of from 10 to 21 mg. Results of single determinations varied from 7 to 23 mg.

Results for the men, 50 to 75 years of age, and for the adult women of all ages were remarkably similar. For these two groups respectively, averages were 9 and 9.3 mg., ranges of individual averages 5 to 15 mg. and 7 to 14 mg., and variations of individual determinations from 4 to 16 mg. and 3 to 16 mg. Thus, with a decline of gonadal activity in the male the excretion of 17-ketosteroids approaches that of the female. It is perhaps significant to mention regarding the 20 females, that 6 healthy young nurses between 19 and 27 years of age excreted a daily average of 10.8 mg., in contrast to the 14 hospital female patients, 17 to 64 years old, who excreted an average of 8.8 mg. in 24 hours.

The individual averages for all men and children were plotted against age, as shown in Figure 1.¹

For each subject the percentage average deviation of individual determinations from his mean value was obtained. In the vast majority of cases it was less than 30 per cent, and never more than 36 per cent. An overall average deviation in percentage, weighted as to the number of determinations per individual, was then calculated. This value was 17.5 per cent. The relative consistency of excretion of 17-ketosteroids from day to day for the normal person is reflected in these figures. Thus, given only a single specimen, the value generally will not deviate by more than plus or minus 30 per cent from the average which would be obtained were

adult women; therefore, the data obtained from them have not been subjected to analysis in relation to age.

RESULTS

The daily values for urinary 17-ketosteroids for the individual subjects were averaged and the values thus obtained were used to calculate the mean and the standard deviation from the mean for each of the groups (Table 1).¹

TABLE 1. DATA ON THE URINARY EXCRETION OF
17-KETOSTEROIDS IN NORMAL SUBJECTS
(Arranged in Relation to Age and Sex)

Age group	Number of		Amount of 17-ketosteroids excreted (mg./day)		Stand- ard deviation σ (from group means)	$\pm 2\sigma$	Coeff. of variation	
	Cases	Determinations	Group average	Range of individual				
				Averages				Determinations
Children under 5	2	3	1.4	0.7- 2.0				
5-12	13	33	3.8	2.4- 5.3	1.0- 8.2	0.9	2.0- 5.6 23.7%	
Males 13-16	3	12	9.4	5.6-14.0	4.0-21.0	3.5	2.4-16.4	
17-34	53	230	18.0	11.0-27.0	9.0-30.0	3.0	16.5%	
17-19	26		17.4			3.4		
20-24	14		17.8			4.0		
25-29	10		19.4			2.0		
30-34	3		20.7			1.4		
35-49	11	34	15.0	10.0-21.0	7.0-23.0	2.8	9.4-20.6 19.0%	
50-75	18	60	9.0	5.0-15.0	4.0-16.0	2.6	3.8-14.2 31.0%	
Females 17-64	20	100	9.3	7.0-14.0	3.0-16.0	2.0	5.3-13.3 21.5%	
Totals	120	472						

Because of the difficulty in securing all the urine from the very young, only three satisfactory samples from two children under 5 years, actually $2\frac{1}{2}$ and 4 years of age respectively, were obtained. The values for 17-ketosteroids excreted in these samples were 0.7, 1.5 and 2 mg. respectively with a mean of 1.4 mg. in 24 hours. In 13 children between 5 and 12 years of age, 33

¹ We wish to thank Mr. Harold Satran, statistician to the Research Unit, for his help in carrying out the statistical calculations.

THYROID COLLECTION OF RADIOACTIVE IODIDE AND SERUM PROTEIN-BOUND IODINE CONCENTRATION IN SENESCENCE, IN HYPOTHYROIDISM AND IN HYPOPITUITARISM*

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IN normal senescence, there is a gradual reduction in basal metabolic rate as well as a decrease in basal body temperature (1). These phenomena have been attributed to decreased activity of the thyroid gland in the aged. Anatomic studies have revealed atrophy of the acini, decrease in the colloid and iodine content, and decrease in the number of dividing cells in the senile gland as compared to the gland of young adults (2). Thus there is both clinical and morphologic evidence to suggest decreased thyroid activity in the senile individual.

Although there has been much speculation as to the cause of senile "hypothyroidism," no experimental data have been presented to elucidate this aspect of the problem. Primary failure of the thyroid to supply adequate hormone has been considered to be the cause of this hypometabolic state (2, 3). Others have postulated that pituitary hypofunction is the primary cause (1).

Benedict's (4) and Boothby's (5) classic studies, thirty years ago, demonstrated that the basal metabolic rate was a useful clinical gauge of thyroid activity. Within the past decade, two more direct assays of thyroid function have been developed, *i.e.* the chemical determination of the concentration of protein-bound iodine in the serum and the determination of the rate of accumulation of radioactive iodide by the thyroid. Salter, Bassett and Sappington (6) and Winkler and associates (7) have demonstrated the very close correlation between the concentration of serum protein-bound iodine and the clinical estimation of thyroid activity. The protein-bound iodine may usually be considered an index of the circulating thyroid hormone. Hamilton and Soley (8) and Hertz and co-workers (9) have shown that the rate of accumulation of radioactive iodide by the

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**AVERAGE AND RANGE OF 24 HOUR URINARY
EXCRETION OF 17 KETOSTEROIDS IN NORMAL
MALES BASED ON 372 DETERMINATIONS IN
100 SUBJECTS**

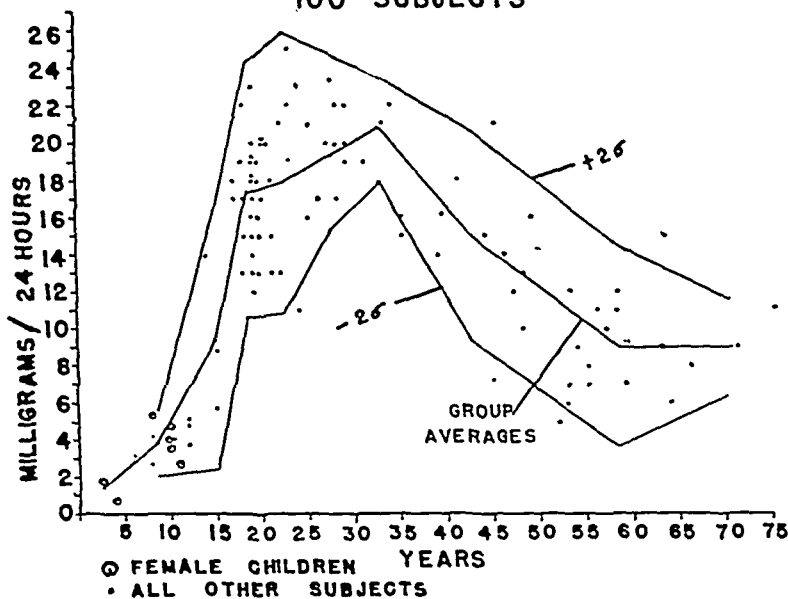


FIG. 1.

several specimens examined. In 15 subjects, the average figures for 17-ketosteroids in three consecutively collected 24-hour samples of urine varied by not more than 2 mg. from similar averages for the determinations through periods of nine days.

SUMMARY

1. The 24-hour urinary excretion of 17-ketosteroids for each of 120 normal subjects, 93 male and 27 female, was determined by a rapid method to establish mean values in relation to age and sex.

2. The average daily excretions of 17-ketosteroids for children, under 5 and between 5 and 12 years of age were 1.4 and 3.8 mg., respectively. For 4 groups of males arranged according to age (13 to 16; 17 to 34; 35 to 49; and 50 to 75 years), the corresponding values were 9.4, 18.0, 15 and 9.0 mg., respectively. The average excretion for females was 9.3 mg.

3. By plotting the individual averages of children and adult males against age, a graph has been constructed which gives the mean and range of the mean 24-hour urinary excretion of 17-ketosteroids.

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iodide uptake by the thyroid. A negative gradient signifies that the initial count at one hour after ingestion of iodide was the greatest and that the count decreased during the ensuing three hours.

The protein-bound iodine¹ of the serum was assayed according to the method of Man and associates (12).

RESULTS

In euthyroidism: The individual accumulation gradients of over 160 patients, who clinically demonstrated no evidence of thyroid dysfunction, are grouped in Tables 1 and 2, according to sex and age. Because there was

TABLE 1. ACCUMULATION GRADIENTS OF EUTHYROID MEN.

Age in years	12-19	20-29	30-39	40-49	50-59	60-69	70 and over
	4.2	6.4	3.4	4.5	2.1	2.9	0.4
	4.8	4.5	2.4	2.3	1.8	1.3	0.4
	3.9	7.4	3.7	1.9	3.7	1.9	0.5
	3.1	3.9	3.4	2.7	3.4	2.3	1.2
	4.0	2.3	2.5	1.6	1.0	3.0	
	10.2	4.5	2.2	11.0	3.6	2.8	
	9.7	1.4	2.2	4.1	2.8	1.2	
	5.5	3.1		2.2	1.2	0.7	
	1.7	2.8		2.6	0.6	1.5	
	0.0	1.2		1.3	1.9	0.7	
	1.7	3.8		3.5	0.6	1.2	
		4.2		3.0	1.6		
		1.4		-0.4			
				0.7			
Mean	4.4	3.8	2.8	2.9	2.0	1.8	0.6
S.E.M.*	0.95	0.53	0.08	0.71	0.31	0.23	0.2

* Standard error of the mean.

considerable scatter within each group and because the number of patients in the seven sub-groups was small, the series was divided into three larger groups. The pubertal group ranged in age from 12-19 years, the young adult group from 20-49, and the elderly adult group, from 50 years up. In Tables 3 and 4, the mean gradients of these three groups are compared.

The effect of aging upon the mean accumulation gradient is presented in Table 3. In both the male and female series, the mean gradients of the

¹ The following ranges are given as a guide to the interpretation of serum protein-bound iodine values: 0-2.9 gamma %, subnormal; 3.0-3.4 gamma %, borderline low; 3.5-6.9 gamma %, normal; 7.0-7.9 gamma %, borderline high; above 8.0 gamma %, above normal.

thyroid can be measured with an externally placed Geiger-Müller counter. Since Morton and associates (10) have demonstrated that most of the iodide accumulated in the normal thyroid is rapidly converted into organically bound iodine, the rate of iodide accumulation by the thyroid can usually be considered indicative of the rate of thyroid hormone synthesis.

Utilizing these two newer methods, the rate of thyroid hormone synthesis and the concentration of circulating thyroid hormone were studied in senescence, in primary hypothyroidism, and in primary hypopituitarism. The rate of radioactive iodide accumulation by the thyroid was determined in 160 hospitalized patients with presumably normal thyroid function, and in 13 patients with hypothyroidism or hypopituitarism. Determinations of the serum concentration of protein-bound iodine were limited to the sera of untreated hypothyroid or hypopituitary patients and of 17 patients clinically free of hypothyroidism or hyperthyroidism, many of whom exhibited a sluggish rate of iodide uptake.

METHODS

The euthyroid subjects were hospitalized patients whose illness had no apparent effect upon their thyroid function; clinically, these patients demonstrated no evidence of thyroid dysfunction. In the others, the diagnosis of hypothyroidism or hypopituitarism was made on clinical grounds. Three hypopituitary patients and one probable hypothyroid patient were kindly sent to us by Dr. F. Albright for this study.

The radioactive iodide, I^{131} , was obtained from the Clinton Laboratories, Oak Ridge, Tennessee. The half-life of this isotope is eight days.

The rate of uptake of radioactive iodide was determined by the method of Stanley and Astwood (11). One-tenth of a millicurie of carrier-free radioactive iodide in approximately 50 ml. of water was administered orally about thirty minutes before breakfast or luncheon. The radioactive iodide was contained in approximately 50 ml. of water. A second 50 ml. of water was taken immediately after the first in order to rinse the radioactive isotope from the glass, mouth, and esophagus. A Geiger-Müller tube, without a lead shield, was placed against the neck as described by Stanley and Astwood (11). A brass shield was placed over the window of the Geiger-Müller tube to block beta radiation from the sweat. Counts, in duplicate, of the gamma radiation emanating from the neck were taken at 1, 2, 3, and 4-hour intervals after the ingestion of the radioactive isotope. The counts per second were plotted as the ordinate and the square root of the time as the abscissa. The slope of this line, approximating a straight line in almost every case, is a numerical expression of the rate of accumulation of iodide by the thyroid and has been termed the *accumulation gradient* by Stanley and Astwood (11). A positive gradient indicates increasing

TABLE 3. MEAN ACCUMULATION GRADIENTS IN YOUNG AND ELDERLY ADULTS. EFFECT OF AGING.

	Mean ±S.E.M.	No. in series	Mean ±S.E.M.	No. in series	Difference of means ±S.E.M.	t Value	Per cent chance that dif- ference is significant
Age in years	20-49		50 and over				
Females	4.8 ± 0.47	35	2.4 ± 0.22	49	2.4 ± 0.51	4.6	99.99
Males	3.2 ± 0.36	34	1.7 ± 0.19	27	1.5 ± 0.43	3.5	99.95

TABLE 4. DIFFERENCE OF MEAN ACCUMULATION GRADIENTS IN MALES AND FEMALES.

	Mean ±S.E.M.	No. in series	Mean ±S.E.M.	No. in series	Difference of means ±S.E.M.	t Value	Per cent chance that dif- ference is significant
Sex	Females		Males				
Age: 12-19	4.1 ± 0.62	6	4.4 ± 0.95	11	0.3 ± 1.1		
20-49	4.8 ± 0.47	35	3.2 ± 0.36	34	1.6 ± 0.59	2.7	99.31
50 and over	2.4 ± 0.22	49	1.7 ± 0.19	27	0.7 ± 0.29	2.4	98.36

In hypothyroidism and hypopituitarism: Eight myxedematous patients were included in this study. Three of these patients had received no thyroid therapy for more than one month prior to the determination of the rate of iodide uptake by their thyroids. Maintenance thyroid medication had not been discontinued in the other 5 patients. The ages, sex, protein-bound iodine concentrations, and accumulation gradients are presented in Table 6.

The radioactive iodide uptake of 5 patients with hypopituitarism was studied; the serum concentration of protein-bound iodine was determined in 3 of these patients. The ages, sex, protein-bound iodine concentrations, and accumulation gradients are presented in Table 7.

DISCUSSION

Technique: Our data agree with Stanley's reports (11, 13) that the accumulation of radioactive iodide by the thyroid approximates a straight line, when the counts per second are plotted against the square root of the time since the ingestion of the isotope. Our accumulation gradients for apparently normal individuals ranged from 0 to 12. This is in close agree-

young adult groups are, statistically, significantly higher than those of the older group.

The difference in gradients between the two sexes is summarized in Table 4. There is no apparent difference between the rate of iodide uptake by the thyroid of the adolescent male and that of the adolescent female in this small series. In contrast, the mean gradient of the females is higher than

TABLE 2. ACCUMULATION GRADIENTS OF EUTHYROID WOMEN.

Age in years	12-19	20-29	30-39	40-49	50-59	60-69	70 and over
	3.2	4.8	5.2	5.1	2.8	3.0	1.6
	6.0	9.7	3.9	5.6	6.3	1.3	2.7
	4.5	4.2	1.5	2.4	1.8	1.7	1.0
	5.0	3.3	2.6	5.1	2.1	1.3	3.3
	1.6	4.2	3.7	5.4	6.6	2.0	2.9
	4.1	8.4	0.6	6.3	5.2	3.1	1.4
		4.5	8.4	7.8	4.9	1.1	1.4
		6.3	10.7	2.8	3.6	1.1	2.4
		5.0	4.4	11.9	1.2	0.9	4.6
		1.8	2.5	1.6	0.0	0.9	
		3.3		9.4	1.8	0.4	
		1.3		1.6	1.8	1.0	
		3.8			0.9	4.8	
					0.7	3.6	
					1.9	2.3	
					1.6	3.0	
					2.9	2.9	
					1.5	5.8	
					1.1	3.1	
					1.6	0.5	
Mean	4.1	4.7	4.4	5.4	2.5	2.2	2.4
S.E.M.*	0.62	0.65	0.95	0.95	0.42	0.33	0.38

* Standard error of the mean.

the corresponding mean of the males in both the young adult and the older adult groups. These differences are probably significant, statistically, since the *t* values are over 2.

As mentioned above, the protein-bound iodine assays were made on the sera of only 17 of the apparently euthyroid individuals. The ages, accumulation gradients and protein-bound iodine concentrations of these patients are presented in Table 5. With one exception, the concentrations of the serum protein-bound iodine were normal despite the low gradients of most of these patients.

ganic or organic iodine. The iodides commonly used as medication, and those iodine-containing compounds used in x-ray examinations of the kidney, lungs, and gallbladder, flood the thyroid with iodide so that falsely low gradients are obtained. Antecedent or concurrent thyroid therapy lowered the gradient in two of our euthyroid patients from 1.6 to 0.5 and from 4.2 to 2.2 respectively. To date, no falsely high rates have been recognized. It is considered likely that some of the low gradients in this series may have been due to undetected antecedent iodide ingestion.

Aging: Our data indicate that there is a decreased rate of iodide uptake in senescence (presumably indicating a decreased rate of thyroxin forma-

TABLE 7. ACCUMULATION GRADIENTS AND SERUM PROTEIN-BOUND IODINE CONCENTRATIONS OF PATIENTS WITH HYPOPITUITARISM

Sex	Age (yrs.)	Serum protein bound iodine (gamma %)	Concurrent thyroid therapy	Accumulation gradient
F	43	—	0	0.7
M	47	1.9	0	1.3
M	47	2.7	+	0.1
F	38	—	+	0.3
F	28	2.3	+	1.4

tion); however, this is associated with a normal level of circulating thyroid hormone. The normal level of blood protein-bound iodine indicates that there is no diminution in the concentration of thyroid hormone reaching the tissues. This suggests that the low rate of thyroid hormone production in senescence is not due to thyroid hypofunction, either primary or secondary to pituitary failure.

It has been demonstrated experimentally that thyroxin administration decreases the thyrotropic hormone (T.S.H.) content of the rat's pituitary to less than 5 per cent of the normal value (14). It has also been demonstrated that administration of thyroid to the normal human temporarily causes thyroid hypofunction (15). As noted here, thyroid medication did lower the gradient in two of our patients. It seems probable that a high level of circulating thyroid hormone depresses T.S.H. production and secretion. That thyroid activity is in large part directly controlled by the pituitary T.S.H. has been well documented. Thus it seems that the concentration of the circulating thyroid hormone indirectly controls the activity of the thyroid. The concentration of the thyroid hormone in the blood depends not only upon the rate of secretion from the thyroid, but also upon the rate of utilization by the tissues or excretion from the body. Decreased utilization of the thyroid hormone by the peripheral tissue

TABLE 5. ACCUMULATION GRADIENTS AND SERUM PROTEIN-BOUND IODINE CONCENTRATIONS OF EUTHYROID PATIENTS.

Sex	Age (yrs.)	Gradient	Serum protein-bound iodine (gamma %)
F	28	9.7	5.0
F	38	8.4	5.6
F	38	0.6	4.1
F	38	1.5	3.8
M	40	1.9	6.4
M	43	1.3	4.0
M	60	2.9	3.6
F	61	1.3	5.0
F	63	0.4	4.1
F	63	1.1	4.2
M	64	1.2	5.3
F	65	1.7	6.1
M	66	1.9	2.4*
F	66	0.9	4.6
M	69	0.8	5.1
F	69	3.0	4.8
F	70	5.5	4.5

* This patient had central nervous system syphilis and clinically did not appear to be hypothyroid.

ment with Stanley's report (13) that the normal range was between 1 and 15.

It would be well to point out that falsely low gradients may be caused by the antecedent ingestion or injection of compounds containing inor-

TABLE 6. ACCUMULATION GRADIENTS AND SERUM PROTEIN-BOUND IODINE CONCENTRATIONS OF PATIENTS WITH HYPOTHYROIDISM

Sex	Age (yrs.)	Serum protein-bound iodine (gamma %)	Concurrent thyroid therapy	Accumulation gradient
F	38	0.8	0	0.5
M	40	—	0	-0.1
F	48	—	0	-0.5
F	49	—	+	0.6
M	56	—	+	-0.1
F	50	—	+	-0.3
F	78	—	+	-0.3
F	59	0.3	+	-0.5

in these cases. Sheehan's (19) pathologic studies in cases of pituitary failure indicate only partial thyroid atrophy in this condition. Thus one would expect that thyroid function would be decreased less in hypopituitarism than in hypothyroidism.

Value of tests: The combined use of the rate of uptake of radioactive iodide and the assay of circulating protein-bound iodine has made possible a dynamic study of thyroid physiology in senescence and in certain hypometabolic states which would have been impossible without these tools. Further combined use of these tests should help to define the role of aberrations of thyroid function in other syndromes associated with abnormal metabolism.

The rather wide scatter of the gradients within each subgroup in Tables 1 and 2 emphasizes the impracticability of using the gradient as the sole means of diagnosing thyroid abnormality. However, when used in conjunction with the assay of circulating protein-bound iodine, it is a test of physiologic as well as of diagnostic importance.

SUMMARY

A study has been made of the rate of uptake of radioactive iodide by the thyroids of 160 apparently euthyroid and 13 hypothyroid or hypopituitary patients. The level of protein-bound iodine of the serum was determined in selected cases.

The causes of a low rate of iodide uptake are discussed. There is considerable scatter of the uptakes within the euthyroid groups.

The mean accumulation gradient, a measure of the rate of iodide uptake, decreases with aging. This decreased gradient is not associated with a decreased concentration of circulating protein-bound iodine.

In the male, there is a gradual drop in the mean gradient from puberty to senescence. In the female there is a sharp drop in the mean gradient, after the menopause, to low levels. There is no sex difference in the mean gradients at puberty; however, after puberty, the mean gradients in the female are consistently higher than in the male.

The accumulation gradients tend to be low in hypothyroidism and in hypopituitarism; in these diseases, they are associated with a subnormal concentration of serum protein-bound iodine.

A mechanism, whereby the peripheral tissue may regulate thyroid function, is postulated and the possible influence of the sex hormones upon peripheral utilization of the thyroid hormone is discussed. Our data suggest that the decreased activity of the thyroid gland in senescence is primarily associated with decreased peripheral utilization of the hormone.

The combined use of the rate of uptake of radioactive iodide and the assay of the circulating protein-bound iodine offers a valuable method for the study of abnormal metabolic states.

might tend to elevate temporarily the concentration of circulating hormone. This presumed temporary elevation of the concentration of circulating thyroid hormone might depress T.S.H. production and thus decrease thyroid activity. This lowered thyroid hormone synthesis would result in decrease of the temporarily elevated circulating hormone level to normal. Thus a normal circulating hormone concentration may be maintained when there is a decrease in the peripheral utilization of thyroid hormone, by means of decrease in thyroid activity. It is suggested that the thyroid hypofunction of senescence may be caused by such a postulated homeostatic mechanism of maintaining a normal level of circulating hormone. This agrees with Thewlis' (1) conclusion that the thyroid atrophy of senescence is not associated with pituitary hypoplasia and is probably due to decreased requirement of the end organ.

Thyroid function may thus be under the control of its end organ, the peripheral tissue, as well as being under the control of its master gland, the pituitary. Sayers and Sayers (16) have postulated a similar control of adrenal cortical function by the peripheral tissue and the pituitary.

If this theory is true, it would indicate that the senile patient with myxedema might need less thyroid replacement therapy than do younger hypothyroid patients. Mueller-Deham and Robson (17) have reported that in the senile hypothyroid patient, the effective dose of thyroid extract is usually smaller than in earlier age groups.

Sex differences: Hyperthyroidism has long been recognized as predominantly a disease of women. It is therefore of interest to note that, after puberty, the rate of radioactive iodide uptake is greater in the female than in the male. It is possible that the more active thyroid gland during the adult female's active sex life is associated with increased peripheral utilization of the thyroid hormone in the presence of the sex hormones. It has been demonstrated that the basal metabolic rate of the hypopituitary individual may be raised by testosterone therapy alone (18). Perhaps the sex hormones, by speeding up body metabolism, may increase the peripheral utilization of thyroxin. The sharp fall of the mean accumulation gradient after the menopause in the female is consistent with this theory. Studies of the effect of sex hormones upon the accumulation gradient are contemplated in order to test this theory.

Hypothyroidism and hypopituitarism: The gradients were low in every case of hypothyroidism and in 4 of the 5 cases of hypopituitarism; it was normal in the fifth hypopituitary patient. Although the gradients seem to be lower in the untreated hypothyroid patients than in the untreated hypopituitary patients, more data are necessary before any conclusion can be reached as to their value in the differential diagnosis of these two conditions. The serum protein-bound iodine levels were definitely below normal

DECREASES IN BLOOD EOSINOPHILIC LEUKOCYTES AFTER ELECTRICALLY INDUCED CONVULSIONS IN MAN

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THE data of earlier work (1, 2, 3, 4, 5, 6) indicate the occurrence of strong stimulation of the adrenal cortex during and after convulsions induced electrically in the treatment of mental disease. The possible importance of increased secretion of some steroid hormones in causing the beneficial response to electroconvulsive therapy is suggested by clinical studies on the effects of testosterone in large doses in patients in relapse after having recovered from mental disease as a consequence of shock therapy (7).

The steroid hormones are known to have several different functions. In an attempt to gain insight into which of these functions might be important in relation to the beneficial effects of shock therapy, it was decided to study the blood eosinophil count in patients given this form of treatment.

MATERIAL AND METHODS

Twenty-one patients, ranging in age from 22 to 65 years, were studied; 14 were women. The diagnoses were various (Table 1). Some of the patients had been ill for long periods but none was more than slightly deteriorated. In the case of the patients with schizophrenia, the clinical course was variable but none of the patients was entirely well for any prolonged period. The patients with manic-depressive psychoses, on the other hand, had periods of complete normality of some duration; in these instances the duration of the present attack is indicated in parentheses in Table 1.

The method described by Forsham and associates (8) was used. A sample of venous blood was taken immediately before a convulsion was induced and again four, eight and, in a few instances, twenty-four hours later. The schedule of treatments was determined by clinical considerations. Forty-six experiments were performed; an additional study was made on one subject who also was given adrenocorticotrophic hormones.

OBSERVATIONS

The eosinophil counts were within the normal range before shock in all instances but one; one patient (Case 6) had an unexplained eosinophilia of 11 per cent so that his eosinophilic cells numbered 2000 per cu.mm. of

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blood. After electro-shock was given, decreases in eosinophilic cell count were found at the end of four hours in all patients but 2 (Cases 5 and 6); in the second of these, a fall of 60 per cent occurred at the end of eight hours after the treatment. In 3 instances (Cases 6, 11, and 15) the changes found at the end of eight hours were larger than those at the end of four hours after treatment, but the differences were not significant in two of these studies (Cases 11 and 15). Of the 13 measurements made at the end of twenty-four hours after treatment, the changes were more marked at this

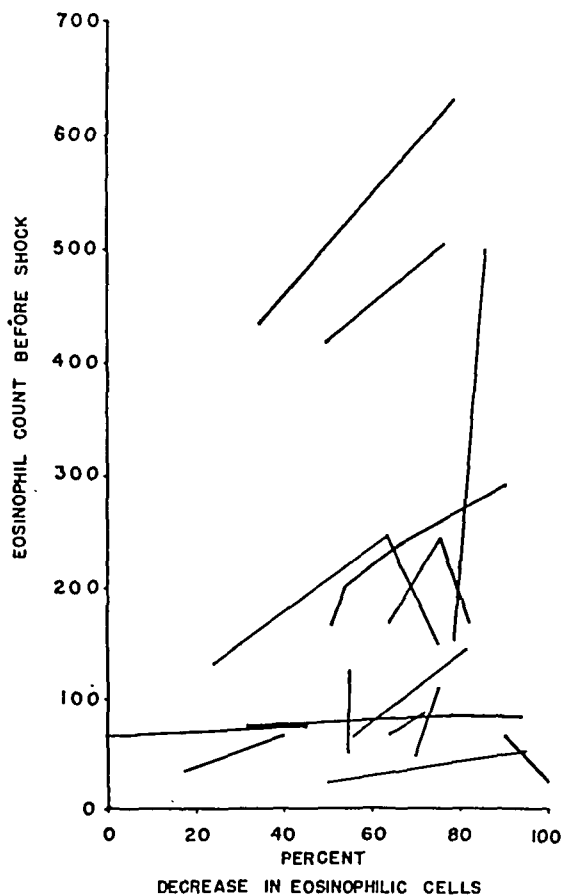


FIG. 1. Relation between initial eosinophilic cell count and magnitude of decrease after electrically induced convulsions.

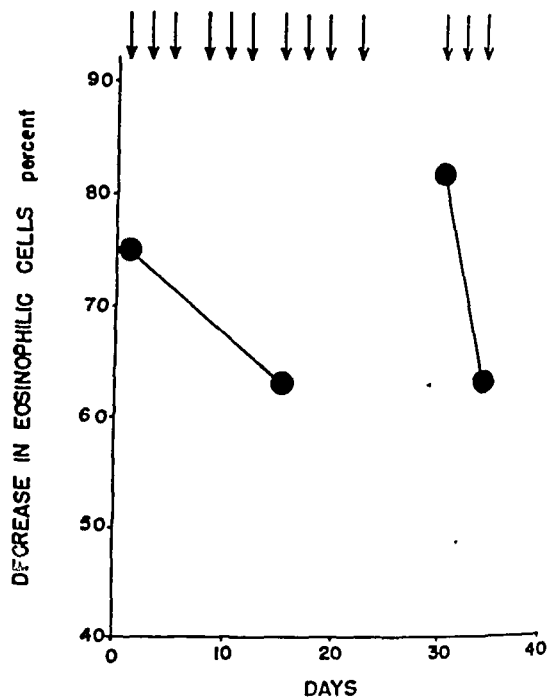


FIG. 2. Recovery of eosinophilic cell response to electroconvulsive therapy after a period of rest. Arrows indicate shocks.

time than at eight hours in 7 (Cases 3, 8, 12, 16, 18, 20) and were the same in 2 (Cases 8 and 11); indeed in 2 experiments the decrease in blood eosinophilic cells was slightly greater at twenty-four than at four hours after the treatment (Cases 6 and 11).

The amount of decrease in eosinophilic cell count after electroshock varied (Fig. 1). All patients studied on the occasion of the first treatment showed decreases in counts of at least 63 per cent irrespective of diagnosis and duration of illness (Cases 1, 2, 11, 12, 13, and 18). When treatments

TABLE 1

Case No.	Age yrs.	Sex	Diagnosis	Total duration	Per cent decrease in eosinophils			Number of shock treatments	Schedule of shock treatments
					in 4 hours	in 8 hours	in 24 hours		
1	57	F	Schizophrenia, paranoid	25 years	100 100 91	100 — —	88 — —	1 10 14	Nine, given three times weekly; a rest of 22 days, then three times weekly.
2	47	M	Schizophrenia, catatonic	6 months	94 50	62 25	— —	1 18	Three times weekly.
3	22	F	Schizophrenia, catatonic	2 years	75 48	0 0	38 —	2 5	Three times weekly.
4	31	M	Schizophrenia, hebephrenic	8 years	75 24 75 64	50 0 — —	— — — —	6 7 11 13	Seven, given once weekly; a rest of 11 days, then three times weekly.
5	30	F	Schizophrenia, catatonic	9 years	45 0	8 0	0 —	6 28	Six, given three times weekly. Then 20, twice weekly.
6	30	M	Schizophrenia, hebephrenic	14 years	0	60	49	11	Three times weekly.
7	36	F	Schizophrenia, paranoid	18 months	83 79	52 —	— —	16 17	Ten, given three times weekly; then one, every 2-3 weeks.
8	50	F	Schizophrenia, paranoid	6 years	18 40	0 11	0 40	27 32	One every five days.
9	25	F	Schizophrenia, paranoid	18 months	77 33	50 0	— —	45 49	Three weekly.
10	49	F	Schizophrenia, paranoid	13 years	75 70	20 —	— —	78 83	One weekly.
11	43	F	Manic-depressive psychosis, depressed.	8 months	73	78	78	1	
12	48	M	Manic-depressive psychosis, depressed.	2 years (5 months)*	66 89 53 51	0 0 35 —	19 33 — —	1 10 14 16	Ten, given three times weekly; then 4, twice weekly; then 2, once weekly.
13	49	F	Psychoneurosis, reactive depression.	1 year	95	55	—	1	
14	52	M	Manic-depressive psychosis, depressed	3 years (3 months)*	72 64	36 —	— —	11 14	Nine, given three times weekly; then 5, once weekly.
15	60	F	Manic-depressive psychosis, depressed.	6 years (6 weeks)*	93 33 77	38 41 43	— — —	4 7 8	Three times weekly.
16	52	F	Manic-depressive psychosis, depressed	3 weeks	56 81	15 13	0 33	2 8	Two, given once daily; then 7, given three times weekly.
17	47	M	Manic-depressive psychosis, manic	3 months	75 63 82 63	49 15 — —	— — — —	1 7 11 13	Ten, given three times weekly; then a rest of a week; then three times weekly.
18	50	M	Psychoneurosis, reactive depression	3 months	77	48	85	1	
19	50	F	Psychoneurosis, reactive depression	2 years	55 55	30 0	— —	6 8	Three times weekly.
20	60	F	Involutional psychosis, melancholia	18 months	70	0	4	9	Three times weekly.

* Duration of present attack.

DISCUSSION

The results of the present study afford additional data supporting the concept that the adrenal cortex is stimulated during the course of electroconvulsive therapy. The decrease in eosinophilic cell count interpreted as evidence of adrenal cortical activity occurred after electrically induced convulsions in patients irrespective of diagnosis or of duration of illness; there was no correlation between the magnitude or persistence of the eosinophil response and the degree or persistence of clinical improvement resulting from the shock treatments given.

The response of the eosinophilic cells decreased with frequently repeated shocks in a manner reminiscent of the findings of Hills and associates (9) after daily injections of adrenocorticotrophic hormone. However, the response returned in the present study after rest periods of ten or more days.

Available evidence indicates that the eosinophilic cell response is consequent to the action of 11-oxysteroids (8, 9, 10). It is evident from the present data that the steroid hormone responsible for the decrease in blood eosinophilic cells after electrically induced convulsions does not give rise to the clinical remission of mental disease secured by shock therapy. Accordingly, it must be concluded that increased production of the 11-oxysteroid hormones does not give rise by itself to the beneficial effects of shock therapy. A previous study (4) ruled out the 3-ketosteroids as effective in this direction. Clinical observations (7) suggest that the hormones which may be responsible for the beneficial effects of shock therapy have anabolic effects. Further studies of the last-named steroid hormones in regard to mental disease are clearly indicated.

SUMMARY AND CONCLUSIONS

Patients given electroconvulsive therapy for mental disease exhibit decreases in blood eosinophilic cell counts irrespective of diagnosis, duration of disease and clinical response to the shock treatment. The change in eosinophilic cell count tends to become less marked when treatments are given frequently and may return to its former level after a rest period of more than ten days.

In one instance the effect of adrenocorticotrophic hormone (Armour) was compared with that of shock therapy, both given over a period of several weeks. The hormone caused a marked eosinophilic cell response but no clinical improvement whereas the convulsions caused a lesser eosinophilic cell response but resulted in clinical remission.

It is concluded that increased production of 11-oxysteroids, considered to be responsible for the eosinophilic cell response, is not the cause of clinical remission in patients given electroconvulsive therapy.

were continued at frequent intervals, *i.e.*, every five days or more often, the amount of decrease in eosinophilic cell count after each shock lessened (Cases 1, 2, 3, 9, 14, and 17). In the case of patients given treatments once a week, insignificant lessening of the response of the eosinophilic cells occurred in two instances (Cases 7 and 10) and a significant lessening was found in one (Case 4).

Recovery of the eosinophilic cell response occurred after rest periods of ten days or more (Fig. 2). This was clearly indicated in two patients (Case 4 and 17) and suggested by the data in one other (Case 1). The finding of a greater response after the eighth than after the second treatment in one patient (Case 16) is probably due to the fact that the second shock followed the first by only one day, whereas all the other shocks given this patient were separated by intervals of two or three days. The small response found after the seventh treatment in Case 15, followed by a large change after the eighth is consequent to the fact that the administration of the treatment caused only a brief period of unconsciousness and no convulsion on the occasion of the seventh shock.

TABLE 2. DECREASE IN BLOOD EOSINOPHILIC CELLS
4 HOURS AFTER TREATMENT

Case 21 (age 65; involutional psychosis, melancholia)

Date	Decrease per cent	Type of treatment
5-28-48	93	Adrenocorticotrophic hormone, 10 mg.
6-16-48	63	First shock
6-28-48	68	Sixth shock
7-2-48	54	Eighth shock

One patient (Case 21, Table 2) was given 0.6 Gm. of adrenocorticotrophic hormone¹ in a period of eighteen days. On the first day of this period the injection of the hormone resulted in a decrease of eosinophilic cell count of 93 per cent; at the end of the eighteen days of treatment the patient was not improved clinically and had lost ten pounds in weight. She was then given eight electroconvulsive treatments over a period of sixteen days; during the course of three of these treatments, studies of the eosinophilic cells showed a lesser decrease than after the administration of hormone (Table 2). She gained six pounds and was discharged clinically well after these treatments.

¹ This was Adrenocorticotropin, Lot G-59703, kindly supplied by Dr. John R. Mote of the Armour Laboratories.

THIOCYANATE GOITER WITH MYXEDEMA

REPORT OF TWO CASES*

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WITH the use of the thiocyanates as therapeutic agents in the treatment of hypertension, thiocyanate goiter has been well established as an entity. The existence of clinical myxedema in association with thiocyanate goiter has been reported. Wald and associates (1) were among the first to report on the formation of a goiter and a decreased metabolic rate in patients receiving thiocyanates for hypertension. Foulger and Rose (2) reported one case in which a goiter and clinical signs of myxedema developed during the course of treatment with thiocyanate. In a case reported by Kobacher (3) myxedema developed, with classical physical findings and a basal metabolic rate of minus 30 per cent. Rawson and co-workers (4) reported two cases, both with goiters and both having levels of protein-bound blood iodine characteristic of myxedema. A biopsy specimen from one patient was reported as revealing an extremely hyperplastic thyroid with marked papillary overgrowth. Potter (5), reporting on the pathology of two goiters (undoubtedly of thiocyanate origin) surgically removed, found brilliantly-staining colloid and no papillary infoldings into the acini. Preoperatively, one goiter was suspected of being carcinomatous and the other of being an acute swelling due to hemorrhage into an adenoma. Motley's (6) patient, to whom thiocyanate had been given for less than a year, had a goiter and a "general appearance quite suggestive of myxedema." Fahlund (7) reported a painful enlargement of the thyroid gland as a manifestation of thiocyanate sensitivity.

A study of the production of thyroid hyperplasia and some of the other abnormal thyroid changes which occur in rats made goitrous by thiocyanates has been accomplished in several laboratories. Astwood (8) included thiocyanate with a large group of antithyroid drugs and reported its hyperplastic action on the thyroid. Rawson *et al.* (9) produced goiters in rats on an iodine-deficient diet followed by addition of potassium thiocyanate to the drinking water. Four hours before killing the rats, the

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blood pressure 170/100. The basal metabolic rate was minus 20 per cent four days after arrival on the ward. Routine urine and blood studies gave normal results. The blood cholesterol was 198 mg. per cent, NPN 26 mg. per cent, and total protein 6.6 Gm. per cent. An electrocardiogram revealed changes consistent with hypertensive and coronary artery heart disease.

Investigation of the "nerve medicine" revealed it to be a proprietary drug containing sodium thiocyanate, 4 Gm. per 100 cubic centimeters. Calculations showed that the patient had taken between 0.2 and 0.6 Gm. daily for two and one-half years. A blood level equivalent to 5.5 mg. per cent of KSCN was found two weeks after the drug had been stopped. (Our laboratory reports all thiocyanate in blood as KSCN equivalents.)

During seven days on the ward a definite decrease in the amount of periorbital edema was noted as well as a marked increase in the patient's activity. A second protein-bound blood iodine taken on October 29, 1947, was 3.4 gamma per cent and a second radioactive iodine excretion was 30.4 per cent (Table 1).

The patient was seen in the Thyroid Clinic on November 12, 1947, at which time he had no complaints other than slight cold intolerance. Only very slight periorbital edema remained, the speech seemed normal and the thyroid gland had decreased in size and was of firmer consistency. The basal metabolic rate was minus 13 per cent.

Case 2: Mrs. H. A. MGH No. 420648, a 64-year-old white widow was referred on September 19, 1947, to the Thyroid Clinic from the Out-patient Department of the Massachusetts General Hospital because of a nodular goiter.

She was first seen in the Medical Clinic in September 1943 with a nine-year history of hypertension. At this time, her blood pressure was 220/115. The blood cholesterol was 275 mg. per cent and there was described a single 2-centimeter nodule in the right lobe of the thyroid. Phenobarbital was prescribed, and she was followed until February of the following year.

She was next seen in the Medical Clinic in July 1947, where her mild cardiac decompensation responded to digitalis. Her blood pressure was 200/90. On August 12, without our knowledge, her private physician prescribed KSCN capsules, 0.2 Gm. daily. On August 18, the blood pressure was 175/75, the blood NPN was 18 mg. per cent, the blood cholesterol 250 mg. per cent, and the blood counts and urinalysis were essentially normal.

On September 19, she was referred to the Thyroid Clinic for the first time. Her complaints were recent onset of deafness and intractable constipation. The basal metabolic rate was minus 11 per cent and the pulse rate 60. There were no other signs of hypothyroidism and the gland was essentially normal except for a 2-centimeter nodule in the lower right lobe.

On November 5, 1947, she again returned to the Thyroid Clinic complaining of deafness and intractable constipation. In addition to these, she had noted marked cold intolerance, absence of perspiration, and dryness of her skin. She presented, on physical examination, rather marked facial and periorbital edema, dry skin, and cold extremities. She was distinctly pale and abnormally hoarse. The palpable thyroid tissue was little changed from her first visit. A blood protein-bound iodine was 0.6 gamma per cent (Table 1). A diagnosis of early myxedema was made. At the time of this visit it was learned that she had been taking thiocyanate capsules. However, the patient stated that she had taken the capsules for only five weeks after they were first given to her in August, and that she had had none since her first visit to the Thyroid Clinic on September 19.

On November 20, a tracer of radioactive iodine was administered and 33 per cent was excreted in the urine. On November 22, a serum thiocyanate level was 4.9 mg. per cent

source of KSCN was removed and they were given radioactive iodine. The goiters produced by KSCN took up an average of 80 per cent, and the control glands 50 per cent of this material. The authors concluded that KSCN inhibited formation of the thyroid hormone, which resulted in hyperplasia of the thyroid and an increased avidity for iodine. On the other hand VanderLaan and Bissell (10) reported that the action of thiocyanate is one which prevents the uptake of iodine by the thyroid, but that this action can be overcome by giving an excess of iodine. Wolff *et al.* (11), reporting on this mechanism, found that as long as the blood level of KSCN remained high, the thyroid was prevented from taking up iodine. The high level of KSCN was shown by these investigators to exert this blocking effect both *in vitro* and *in vivo*. They concluded that there was rapid elimination of the KSCN from the blood, probably complete in twenty-four hours, in the rat. They would explain Rawson's results on this basis, by saying that the KSCN blood level fell during the four hours the rats were not receiving the drug and iodine was able to enter the hyperplastic gland.

CLINICAL MATERIAL

We are reporting two more patients who developed myxedema and goiters as the result of taking thiocyanate.

Case 1: J. H., MGH No. 113448, a 51-year-old white business man entered the Eye Clinic of the Massachusetts Eye and Ear Infirmary on September 9, 1947, complaining of puffy eyelids. A mild conjunctivitis was noted and treated. On a return visit, three weeks later, because he complained of persistent puffiness of the eyelids and enlargement of his neck, he was referred to the Medical Service of the Massachusetts General Hospital for study.

On October 15, 1947, he was seen in the Thyroid Clinic where further history revealed cold intolerance, decreased perspiration, lassitude, and somnolence, and a gain in weight of 8 pounds during the preceding four to six months. He stated that he had been taking a liquid "nerve medicine" for the past two and one-half years because of high blood pressure.

Physical examination, at this time, revealed a well developed, somewhat obese, jolly male in no evident distress. The skin was warm and dry. There was moderate periorbital edema without conjunctivitis, and examination of the fundi revealed minimal arteriovenous nicking. The tongue was wide and thick, the speech slow, and the voice of deep pitch. The thyroid gland was enlarged to 2 or 3 times normal size and was soft without nodules, bruit or thrill. The heart was of borderline size, the pulse rate was 60 and the blood pressure was 168/104. A smooth nontender liver edge was felt 2 finger breaths below the right costal margin. Laboratory data showed a basal metabolic rate of minus 38 per cent, blood cholesterol 298 mg. per cent, protein-bound iodine 1.7 gamma per cent (normal 3.5 to 7), and a urinary excretion of radioactive iodine of 50 per cent (probably normal). The patient was instructed to stop taking the "nerve medicine" at the time the radioiodine was started.

He was admitted to the medical wards on October 24, 1947, with a diagnosis of myxedema with goiter. At this time the physical examination was essentially the same except that the face was ruddy in color, the skin somewhat moist, the pulse rate 80, and the

Case 1 will return to normal as it did in Case 2, in which instance the gland required less iodine as it returned to the normal production of hormone. This production was reflected in the rising protein-bound iodine of the blood.

The first patient did not develop myxedema until he had taken thiocyanate for two years. The second patient developed myxedema after one month of thiocyanate therapy. The reason for this difference is not evident.

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and the blood protein-bound iodine had risen to 3.3 gamma per cent. On December 12, the blood iodine level was 4.3 gamma per cent, the urinary excretion of a second radioactive iodine tracer dose was 48 per cent, and a blood KSCN level was 1.8 mg. per cent.

At the present time, five months after she was first seen in the Thyroid Clinic, she has none of the former symptoms or signs of hypothyroidism and is subjectively well.

TABLE 1

Patient No.	Length of time thiocyanate administered	Date thiocyanate omitted	Date of laboratory data	Basal metabolic rate %	Protein-bound blood iodine, gamma %	Urinary excretion of radioactive iodine, (per cent of administered dose)
1	30 months	10/15/47	10/15/47	-38	1.7	50
			10/28/47	-20		
			10/29/47		3.4	30.4
			11/12/47	-13		
2	5 weeks	9/19/47	9/19/47	-11		
			11/ 5/47		0.6	
			11/20/47			33
			11/22/47		3.3	
			12/12/47		4.3	48

DISCUSSION

Two patients, during thiocyanate treatment for hypertension, developed clinical myxedema with levels of basal metabolism and protein-bound blood iodine characteristic of this disease. After thiocyanate therapy was discontinued, the myxedema disappeared and the basal metabolic rate and the blood protein-bound iodine returned to normal.

The uptake of radioactive iodine in the first case was at first normal, then high. Apparently the level of thiocyanate in the blood was high enough to block partially the radioactive iodine uptake at the time of the first dose, but as the level fell, the depleted hyperplastic gland was able to take up iodine in larger quantities. It can be anticipated that iodine uptake in

second admission, nausea and vomiting had occurred without pain or hematemesis. There had been recent constipation, but no hemorrhoids or melena. Marked weakness was reported.

Physical examination: The patient was a well developed, fairly well nourished, white man who appeared acutely ill. He had a paroxysmal cough productive of bloody sputum. Extreme tenderness and pain accompanied movement of all extremities, particularly the left shoulder and arm. Positive findings in the mouth and throat were dental caries and enlarged tonsils. On examination of the chest there was tenderness of the nipples but no enlargement of the breasts. Breath sounds were decreased bilaterally but there were no rales or alteration of fremitus. The heart was normal in size, the sounds were clear, and the rate was 100 with irregular rhythm. An abdominal mass the size of an orange was palpable in the right upper quadrant laterally, with tenderness present over the mass and tenderness in the left lower quadrant near the ileum. No other organs or masses were palpable. The genitalia were normal in size and appearance. No blood or discharge could be expressed from the urethra, but the prostate was slightly enlarged and soft. A large soft fixed lymph node was palpable in the left axilla. Except for absence of the left triceps, biceps and wrist jerks, the neurologic findings were normal.

*X-ray and laboratory data: First admission (Mt. Sinai Hospital):*¹ An x-ray picture of the chest was interpreted as showing a metastatic malignant lesion. An intravenous urogram demonstrated ptosis and slight enlargement of the left kidney. No additional information was obtained from cystoscopic examination, urine examination for acid-fast organisms, x-ray examination of the left shoulder girdle, left arm and gall bladder, gastro-intestinal series and barium enema. Bronchoscopy in August 1947 was reported as showing "a compression of the right lateral and posterior walls of the mid-trachea by an external mass which cut the lumen by one-third. The overlying mucous membrane was normal; otherwise the bronchoscopic findings were normal." Bronchial secretion was devoid of acid-fast organisms and malignant cells. The Friedman test was reported positive August 18, and the positive finding confirmed on a second test. Study of urinary hormone output on September 2 showed the patient was excreting 50,000 mouse units of Prolan A per 100 ml. of urine.

Second admission (Philadelphia General Hospital): X-ray examination of the chest showed metastatic malignancy throughout. Additional x-ray studies revealed early metastatic lesions of the lower left humerus and an enlarged right kidney, but no evidence of metastases to the skull, vertebrae or pelvic bones. An intravenous pyelogram demonstrated impaired function of the left kidney and a large irregularity suggesting primary malignancy. The right kidney functioned normally. Blood studies revealed 2.78 million erythrocytes and 10,850 leukocytes. There were 77 per cent polymorphonuclear cells, 21 per cent lymphocytes, 1 per cent monocytes and 1 per cent eosinophiles. Sugar was 81 mg. per cent, urea nitrogen 14 mg. per cent, and total protein 6.0 Gm. per 100 ml. with an A/G ratio of 3.4:2.6. The CO₂ combining power was 17.6 mEq. and Cl (NaCl) 104.5 mEq. per liter. A Kline test was negative. Urinalysis showed a specific gravity ranging from 1.009 to 1.017. There was an occasional 1 plus albuminuria, and the sediment showed 10 to 15 erythrocytes per high power field and many leukocytes.

Course: In spite of treatment by bed rest, opiates for relief of severe pain, and an indwelling catheter, the patient followed a rapidly declining course with gradual onset of severe dyspnea, and died October 16, 1947.

¹ I am indebted to Dr. D. Meranze for letting me use these data.

ADRENAL CORTICAL CARCINOMA IN A MALE WITH EXCESS GONADOTROPIN IN THE URINE

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From the Philadelphia General Hospital, Philadelphia, Pennsylvania

TUMORS of the adrenal cortex with symptoms of hypercorticism are infrequent (1-3). Most of the cases reported presented masculinization (in females) or the adrenogenital syndrome (in males or females); a few showed evidence of feminization (in males)(4-12). Adrenal cortical tumors associated with excess gonadotropin seem to be exceedingly rare. A search through the literature revealed only one case (McFadzean) (13):

The 29-year-old patient was admitted because of unexplained fever. He gave a history of symptomless breast enlargement of eighteen months' duration, of weight gain of one year's duration, and of decreased sexual activity and libido of six months' duration. A large tumor mass was palpable in the upper left quadrant. The breasts were well developed, the genitalia were normal, and there was a slight feminine distribution of his hair. X-ray examination showed a soft tissue mass with displacement of the left kidney. The lungs were clear. Urinary estrogens or gonadotropins were not assayed, but a Friedman test was positive. Twenty days after successful removal of the tumor, the Friedman test was found to be negative. Sexual interest and activity returned, and by the forty-third postoperative day the beard was thicker and periareolar hair had grown. The tumor was mostly encapsulated, multilobulated and weighed 1420 Gm. It showed fibrous cores, multiple hemorrhages and light gray areas, and one lobe resembled the "classical hypernephroma." Microscopic examination revealed an adenocarcinoma, the structure resembling that of adrenal cortex with various types of nuclei and some vacuolization.

As only one case was found in the literature, it seemed of interest to report another case in which both Friedman tests and gonadotropin assays were made.

CASE REPORT

Present illness: The patient was a white male, 39 years old at the time of his first hospital admission July 31, 1947. The presenting complaints were cough with hemoptysis, hematuria occurring at the end of micturition, bloody ejaculation and continuous progressive pain in the left arm and both flanks. These complaints had developed over the preceding ten or eleven weeks. Diagnosis of metastatic malignancy was made, although the primary site was not discovered. The patient received deep x-ray therapy (8 series, 200 r) before his discharge on August 22. At the time of the second hospital admission in September 1947, he stated that all symptoms had persisted and progressed in severity except the hematuria. In addition, the breasts had become sore. The flanks, which ached continuously were sore to the touch, and pain in the chest accompanied the productive cough. On being questioned, the patient stated that he had noted continuous right frontal headache and pain in the right eye, but no impairment of vision. Eight days prior to the

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contained a tumor mass approximately 12 cm. in diameter causing extension of this lobe down to the iliac crest. There was a small metastatic nodule near the diaphragmatic portion and several very small nodules throughout the inferior portion of the left lobe. The latter nodules were purplish in color, while the large mass in the right lobe was white. The remaining liver substance was pale brown and somewhat greasy. The portal venous system was normal. Gall bladder and bile ducts were normal. The pancreas contained several small tumor nodules within the body, each approximately 1 cm. in diameter. The duct appeared to be normal. The left adrenal appeared to be normal. The right was changed into a round, firm, well encapsulated mass measuring 3 cm. in diameter. On cut section, it was homogeneously purplish in color and distinctly firm throughout. The thyroid appeared to be normal. The distal end of the left humerus was completely sur-

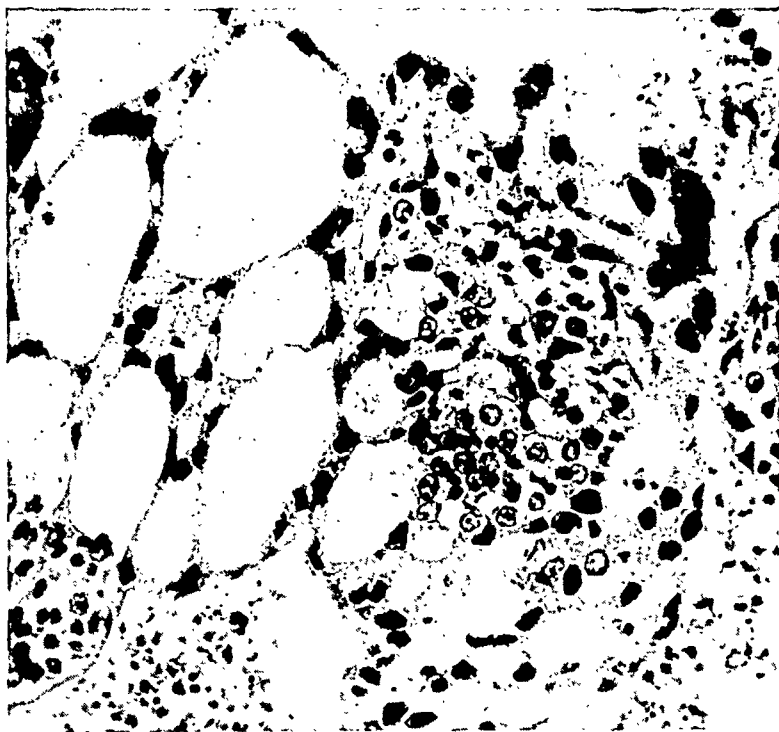


FIG. 2. Portion of tumor forming spaces lined with epithelial cells resembling chorion epithelium. Hematoxylin-eosin, $\times 250$.

rounded by tumor tissue, similar to that described above. It was approximately 1 to $1\frac{1}{2}$ cm. thick in all areas around the distal end of the bone and appeared to replace the periosteum. The underlying bone was rough and irregular. The tendon of the triceps brachii was completely replaced by this mass in its distal portion. The humerus was free below the condyles and below the mid-portion. On section, the tumor did not invade the bone at all, although the medullary substance was soft and of purple color. The remaining bones appeared to be normal. Brain and pituitary were not examined.

Microscopic examination: In some places, such as the lungs (Fig. 1), the tumor formed solid sheets of large polygonal cells with large somewhat vesicular nuclei not unlike the cells of the adrenal cortex. There were occasional giant cells with large hyperchromatic nuclei. There were many mitotic figures. In other places, such as in the liver (Fig. 2), the tumor formed bizarrely shaped spaces often lined with large endothelial-like epithelial

Postmortem examination: The body was that of an extremely cachectic white male. The distal end of the left arm was diffusely swollen. The breasts were grossly normal. The serous membranes were all smooth and glistening and no adhesions or fluid were found. The vessels were normal throughout. There was no arteriosclerosis. The heart weighed 280 Gm. It was flabby and showed slight dilatation of the left ventricle and marked dilatation of the right ventricle. The left lung weighed 1300 Gm., the right 1800 Gm. External surfaces were smooth, blackened and nodular. Throughout the parenchyma of each lobe hard, purplish white nodules, measuring approximately 2 to 3 cm. in diameter, were found. The intervening lung tissue was congested. Bronchi and vessels were clear.

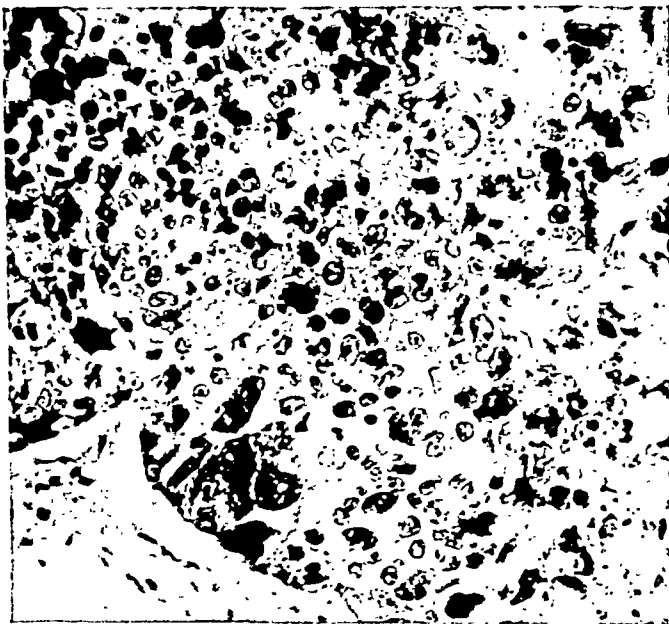


FIG. 1. Portion of tumor forming solid sheets of polygonal cells including giant cells with large hyperchromatic nuclei. Hematoxylin-eosin, $\times 250$.

The spleen weighed 80 Gm. The capsule was smooth. The consistency was soft. The color was purplish. There was no evidence of tumor. The mesentery and mediastinal lymph nodes were enlarged and replaced by tumor tissue. The external nodes were normal. The left kidney weighed 330 Gm., the right 180 Gm. The entire lower pole of the left kidney was replaced by a tumor mass, measuring 8 cm. in diameter. It was round with an irregular surface, and on cut surface was white in color with streaks of purplish red. In the mid-portion of the left upper lobe, a firm, round nodule measuring approximately 2 cm. in diameter was found. It was purplish-red in color but did not show as much firm, white tissue as the one in the lower lobe. The remaining parenchyma was pale and somewhat cloudy. The right kidney contained several small purplish nodules measuring from 1 to 2 cm. in diameter. Renal pelvis were normal except for distortion by tumor masses. The ureters were normal. The urinary bladder was not changed. Both testes were normal in size and consistency. The prostate was normal. The esophagus, stomach and bowel were not changed. The mesentery of the small bowel contained small metastatic nodules approximately 1 cm. in diameter, which were of the same consistency as the tumor tissue elsewhere. The liver weighed 2330 Gm. The interior and lateral portions of the right lobe

SUMMARY

A case of adrenal cortical carcinoma in a man with excess gonadotropin excretion is reported. The patient excreted 50,000 mouse units of gonadotropin per 100 ml. of urine and the Friedman test was positive.

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cells resembling chorion epithelium. The latter formed multinucleated giant cells in places. The small spaces surrounded by these cells contained protoplasmic debris and plasma, while the large ones contained erythrocytes and leukocytes as well. These portions of the tumors, too, contained abundant mitotic figures. All tumors showed more or less extensive necrosis. The one in the right adrenal gland was almost completely necrotic. There was little fat in the tumor cells except in the areas where they underwent necrosis.

The testes were somewhat atrophic. Spermatogenesis was almost completely suppressed. The spleen showed chronic splenitis with a good many plasma cells and eosinophiles. The lungs, liver and kidneys were markedly congested, and there was extensive central necrosis of the liver lobules.

The *final diagnosis* was carcinoma of the cortex of the right adrenal with metastases to lymph nodes, lungs, liver, kidneys, pancreas and left arm. Cause of death was respiratory and circulatory failure.

COMMENT

The case of adrenal cortical carcinoma reported here resembled that of McFadzean's (13) in that the patient was a male, and excess gonadotropin was excreted in the urine; it differed in that it showed no convincing evidence of feminization. In the case of a male reported by Simpson and Joll (11) endocrine assays revealed excess estrogen excretion in the urine, but the Aschheim-Zondek test was negative, and no gonadotropin was demonstrable. These observations seem to show that adrenal cortical carcinomas may be associated with either excess estrogen excretion or excess gonadotropin excretion or both.

The cellular sources of gonadotropins are generally believed to be the chorion epithelium of the placenta and the anterior lobe cells of the pituitary gland. In the case reported here, as in McFadzean's case, we have no evidence that the gonadotropins were produced by either placental tissue or the pituitary gland. In our case, the histologic structure of the tumor resembled that of adrenal cortex in most places, though here and there the cells showed differentiation towards elements resembling chorion epithelium (Fig. 2). In McFadzean's case, the Friedman test became negative promptly after successful removal of the tumor; whereas in cases with excess hypophyseal gonadotropin excretion secondary, for instance, to seminoma testis, successful treatment of the tumor is said not to cause a prompt fall in gonadotropin and the hormone continues to be excreted in the urine in excessive amounts for some time (13). Moreover, McCullagh and Cuyler(14) reported a case of Cushing's syndrome due to a pituitary adenoma in a woman with a positive reaction to the Friedman test. Her symptoms and the gonadotropin excretion all disappeared following denervation of both adrenals and right hemiadenectomy, without therapy to the pituitary gland. These observations seem to indicate that the adrenals may be instrumental in the elaboration of gonadotropic hormones.

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GYNECOMASTIA IN PARAPLEGIC MALES

REPORT OF SEVEN CASES

IRVING S. COOPER, M.D.* AND THOMAS I. HOEN, M.D.

United States Naval Hospital, St. Albans, New York

THE authors wish to report 7 cases of gynecomastia occurring in paraplegic males. These cases are believed to be significant for the following reasons: 1) Out of 32 paraplegic patients on our ward at one time, these 7, or 22 per cent of the paraplegic patients at that time under our observation, showed gynecomastia. 2) All of these patients were young males of military age, and all were paraplegic due to traumatic injuries of the spinal cord. 3) The physiologic implications of this syndrome may be of practical value as well as academic significance.

Our attention was first called to this unusual syndrome by the patients on the paraplegic ward who had singled out one of their number by referring to him as "Mae West." It was then apparent to us that this patient had developed a bilateral enlargement of both breasts, and, in a survey of the 32 patients on the ward at that time, 7 of the patients proved to have either bilateral or unilateral gynecomastia. Several stated that the enlarged breast was tender, and a few of the patients who did not show evidence of gynecomastia at that time believed that they had had enlarged breasts at some time during their illness.

Gynecomastia has been reported in many diverse clinical entities. In 1944 Webster (1) reported that in the years 1939 through 1941, from 6.9 to 8.69 otherwise normal males out of every 100,000 men entering the navy showed gynecomastia. Our incidence of 22 per cent in this series of paraplegics, contrasted to 8 per 100,000 in normal males of the same age group, would appear to be strikingly significant. The finding of gynecomastia in liver disease, particularly cirrhosis, is a well documented observation (2). It has also been reported as a consequence of desoxycorticosterone acetate (DOCA) therapy in both mice (3) and humans (4), in Addison's disease (5), in vitamin deficiency (6), in a case of choriocarcinoma of the testis (7), in a man with bilaterally undescended testes (8), in a case of extra-genital chorionepithelioma (9), and in other diverse disease entities. At first glance, an etiologic common denominator underlying these various disease states which have been reported as being instrumental in producing gynecomastia, is not apparent. However, there are several endocrinologic factors which most of the reported cases might conceivably have in common.

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Edmonson, Glass, and Soll (2) have demonstrated the fact that the degeneration of the liver parenchyma causes an excess of estrogen in the systemic circulation, and thus suggest an endocrinologic basis for the breast hypertrophy in cases of liver disease. Klinefelter, Reifenstein, and Albright (10) reported a syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism, and increased urinary excretion of F.S.H. (follicle stimulating hormone). In these cases the testes were small, measuring about $1.5 \times 1.0 \times 0.5$ cm., the urinary 17-ketosteroid levels varied from relatively normal to definitely subnormal, and the testicular biopsy



FIG. 1.

revealed primarily a hyalinization of the tubules. This syndrome has also been reported by Bettinger and Robinson (11), and more fully by Heller and Nelson (12). Klatser, Salter and Humm (13) reviewed very carefully 48 cases of gynecomastia occurring among prisoners of war who had been suffering from malnutrition. Their clinical studies revealed abnormalities of both the liver and the genitalia in these patients. The same authors (14) reported that these patients suffering from malnutrition, and demonstrating gynecomastia, were found to have significantly lowered 17-ketosteroid levels in the urine. However, urinary estrogen values were in the low normal range, the cortin levels were normal, and there was no apparent increase in the urinary titer of F.S.H. Therefore, at this time, there is no clear cut, general interpretation of all

of the endocrinologic factors in the pathogenesis of gynecomastia. These cases of gynecomastia in paraplegics may serve to add another link in the chain of information leading to an understanding of this entity.

CASE REPORTS

Case I. W.E.M., a 20-year-old private in the Marine Corps, was injured in April 1945, with a resultant paraplegia below the level of the twelfth thoracic dermatome. He had lost 75 pounds during the subsequent four months but by May 1946, had returned to his normal weight. Bilateral gynecomastia was first noted in May 1946 (Fig. 1).

Case II. J.S., a 39-year-old chief carpenter's mate in the Navy, was paraplegic below

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Case II. J.S., a 39-year-old chief carpenter's mate in the Navy, was paraplegic below

the level of the first lumbar segment as a result of a fracture dislocation of the vertebral column incurred in a fall. Bilateral gynecomastia appeared approximately eighteen months following the injury. Incidentally, the patient had had his left testis surgically removed because of a tumor therein. This operation took place overseas during the war, and no pathologic report was obtainable.

Case III. W.W., a 22-year-old corporal in the Marine Corps, was rendered paraplegic by shrapnel wounds of the spinal cord. A unilateral (left) gynecomastia was observed fourteen months later. The patient could not state how long this breast had been enlarged.

Case IV. A. W., a 26-year-old Marine private, was paraplegic below the ninth thoracic segment as a result of a shrapnel wound of the spinal cord. Bilateral gynecomastia appeared approximately ten months following the injury and had been present for three months at the time of observation.

Case V. T.R.K., a 19-year-old private in the Marine Corps, was paraplegic below the sixth thoracic segment as a result of a bullet wound of the spinal cord. Unilateral (left) gynecomastia was first noted fifteen months after the injury.

Case VI. W.W., a 22-year-old seaman second class in the Navy, was rendered paraplegic from wounds received from machine gun bullets. A unilateral (left) gynecomastia was noted approximately sixteen months following this injury. This patient had a recurrent left epididymitis as a result of infection from an indwelling urethral catheter.

Case VII. E.S., Jr., a sergeant in the Marine Corps, had complete motor and sensory loss below the sixth thoracic dermatome, following a motor vehicle accident in which he incurred a compression fracture of several thoracic vertebrae. A bilateral gynecomastia was noted twenty months after this injury.

We were able to obtain single F.S.H. studies on the urine of patients II, IV, and VI. In cases II and VI there was a positive reaction for 96 mouse units in a twenty-four hour specimen, this being a pathologically significant elevation of F.S.H. Measurements of the testes of all 7 patients were in the low normal range. Only 1 of the 7 was able to have ejaculation of semen, and this was free of motile sperm. In only 1 of these cases was malnutrition considered to have been a prominent feature, and 2 of the cases showed testicular pathologic processes, as noted in cases II and VI above.

Possible Pathogenesis of Gynecomastia in Paraplegics

At this time we can do no more than suggest certain hypotheses for the pathogenesis of the breast hypertrophy occurring in these paraplegics. Although the testicular measurements were within low normal limits, it is possible that these cases would fall into the same category as those described by Klinefelter, Reifenstein, and Albright. In 2 of the 3 cases in which urinary F.S.H. titers were obtained, there was a significant elevation of this gonadotropin. In only 1 of the 7 patients was there the power of ejaculation. None of these patients was willing to submit to biopsy of the testis. (All of these patients were engaging in autistic thinking as regards the return of sexual capacity and were unwilling to allow any procedure which might conceivably damage the sexual organs.) Thus it is seen that

at least 2 of these patients showed gynecomastia, aspermatogenesis, and increased F.S.H. excretion. It is not unlikely that further studies on similar patients may reveal these patients to be examples of the Klinefelter-Reifenstein-Albright syndrome secondary to traumatic lesions of the spinal cord.

Salter, Klatskin, and Humm (14) suggested that an involvement of the pituitary-gonadic axis might be the cause of the gynecomastia seen in prisoners of war suffering from malnutrition. It is possible that such an involvement may have caused the gynecomastia in our paraplegic patients. Selye (15) has demonstrated the degenerative changes that occur in the anterior lobe of the pituitary gland in the diseases which act as stimuli for the alarm or adaptation reaction. Spinal cord transection is one of the many stimuli which may produce the alarm reaction (16). Certain it is that these war-wounded paraplegics are subjects in whom the adaptation syndrome may well be expected to follow the severe trauma incurred. The secondary endocrinologic changes which may follow hypopituitarism are hypothyroidism, hypofunction of the adrenal cortex, and hypogonadism (17). It is not unlikely that the adaptation syndrome, with its consequent hypopituitarism, might be instrumental in bringing about hypogonadism, and thereby contribute to the pathogenesis of gynecomastia in paraplegia due to traumatic injuries of the spinal cord.

SUMMARY

1. Seven cases of gynecomastia occurring in young paraplegic males are presented. Four of these patients had bilateral breast hypertrophy and three had unilateral (left) gynecomastia.

2. Some of the theoretical considerations regarding the pathogenesis of gynecomastia in these cases have been presented.

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the level of the first lumbar segment as a result of a fracture dislocation of the vertebral column incurred in a fall. Bilateral gynecomastia appeared approximately eighteen months following the injury. Incidentally, the patient had had his left testis surgically removed because of a tumor therein. This operation took place overseas during the war, and no pathologic report was obtainable.

Case III. W.W., a 22-year-old corporal in the Marine Corps, was rendered paraplegic by shrapnel wounds of the spinal cord. A unilateral (left) gynecomastia was observed fourteen months later. The patient could not state how long this breast had been enlarged.

Case IV. A. W., a 26-year-old Marine private, was paraplegic below the ninth thoracic segment as a result of a shrapnel wound of the spinal cord. Bilateral gynecomastia appeared approximately ten months following the injury and had been present for three months at the time of observation.

Case V. T.R.K., a 19-year-old private in the Marine Corps, was paraplegic below the sixth thoracic segment as a result of a bullet wound of the spinal cord. Unilateral (left) gynecomastia was first noted fifteen months after the injury.

Case VI. W.W., a 22-year-old seaman second class in the Navy, was rendered paraplegic from wounds received from machine gun bullets. A unilateral (left) gynecomastia was noted approximately sixteen months following this injury. This patient had a recurrent left epididymitis as a result of infection from an indwelling urethral catheter.

Case VII. E.S., Jr., a sergeant in the Marine Corps, had complete motor and sensory loss below the sixth thoracic dermatome, following a motor vehicle accident in which he incurred a compression fracture of several thoracic vertebrae. A bilateral gynecomastia was noted twenty months after this injury.

We were able to obtain single F.S.H. studies on the urine of patients II, IV, and VI. In cases II and VI there was a positive reaction for 96 mouse units in a twenty-four hour specimen, this being a pathologically significant elevation of F.S.H. Measurements of the testes of all 7 patients were in the low normal range. Only 1 of the 7 was able to have ejaculation of semen, and this was free of motile sperm. In only 1 of these cases was malnutrition considered to have been a prominent feature, and 2 of the cases showed testicular pathologic processes, as noted in cases II and VI above.

Possible Pathogenesis of Gynecomastia in Paraplegics

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THE USE OF BISMUTH SALTS IN THE TREATMENT OF SPORADIC GOITERS

MANUEL VILLAVERDE, M.D.*

THE therapy of simple goiter is practically limited nowadays to the use of iodine or desiccated thyroid and, finally, to surgical removal for cosmetic reasons. The purpose of this communication is to call attention to observations on another method which may conceivably prove to be of some value.

While treating some patients with bismuth salts in the routine treatment for syphilis, it was noted that a goiter, an incidental finding, was noticeably reduced in size during the treatment. Following this observation, a number of patients with goiter but without syphilis were deliberately treated with bismuth salts. In some instances there was a striking reduction in size, almost to normality. These results have been published in a preliminary report.¹

No data having direct bearing on this subject could be found in the medical literature. The excellent studies on bismuth, of Sollman and coworkers, did not mention possible actions of bismuth on the thyroid; but since this gland has a large vascular supply, it may be that the amount present in the blood represents the amount in the gland. Bismuth is stored principally in the kidneys, in the amount of 3.33 mg. of bismuth per 100 Gm. of fresh tissue; the figure for the liver is 0.68 mg. and for the blood, 0.05 mg.

A long-standing enlargement of the thyroid which is symmetrical and firm and does not produce symptoms of hyperfunction, is very suggestive of simple goiter, particularly when this condition occurs also in other members of the family. Nodular enlargement may or may not be adenoma, because these irregular masses can also be in the nature of a simple goiter. Clinically, it is not very important to differentiate simple from nodular goiter, for both lack hyperfunction and their harm is merely local; yet both can assume hyperfunction in some instances, and both can disappear spontaneously.

MATERIAL AND METHODS

The treatment at first was confined to the simple and nodular goiters and later it was extended to include other thyroid conditions including hyperthyroidism. In these latter instances, the bismuth therapy invariably appeared to have no effect on the hyperthyroidism, but when this condition

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¹ Villaverde, M.: *Bocios tratados con sales de bismuto, Vida Nueva* (Havana), 58: 233 (Dec.) 1946.

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TABLE I

Case No.	Family Background		Personal history	Syphilis	Serpologic tests	Endocrine symptoms	Goiter (diffuse or nodular)	Basal metabolic rate, %	Body constitution	Circulatory symptoms	Additional diagnoses		Number of series	Number of injections	Results	Remarks
	Syphilis	Goiter														
1	+	+	+	+	+	+	D	+1	III*	-	Syphilitic chancre	3	60	+	++§	
2	-	-	-	-	-	-	D	+34	II	-		3	60	+	++	
3	-	-	-	-	-	-	D	-19	II	+	Hyperthyroidism	2	40	+	++	
4	-	+	+	+	+	+	D	+9	II	-		1	20	+	++	Normality after 15 injections.
5	-	-	-	-	-	-	D	+25	III	+	High blood pressure	2	40	+	++	Goiter developed after treatment for syphilis.
6	?	-	-	-	-	-	D	±0	II	+		1	20	+	++	
7	?	-	-	-	-	-	D	-7	II	+		2	39	+	++	
8	-	-	-	-	-	-	D	+23	II	+	Hyperthyroidism	1	20	-	-	Intolerance to bismuth. Developed tuberculosis.
9	-	-	-	-	-	-	D					2	30	-	-	
10	-	+	-	-	-	+	D	-33	II	-	Hyperostosis frontalis interna; anxiety neurosis	2	40	-	-	
11	+	+	+	-	-	-	N	-22	II	-	Backache	1	20	-	-	Iodine had failed previously.
12	-	+	+	-	-	-	N	-5	II	-		2	40	+	++	Relapse after 10 weeks
13	-	-	-	-	-	+	N	+21	II	+	Hyperthyroidism	2	40	+	++	Discomfort disappeared on swallowing.
14	+	-	-	-	-	+	N	-23	II	-	Hyperestrogenism	2	40	+	++	Normality before the end of the second series.
15	-	?	?	-	-	-	N	-13	II	-		2	40	+	++	
16	-	-	-	-	-	?	N	+17	II	+	High blood pressure	2	40	+	++	
17	-	-	-	-	-	-	D	+11	II	+	Graves' disease	1	20	-	-	
18	-	+	-	-	-	-	D	-6	II	+	Basedow neurosis	1	20	-	-	

* III = pyknic type; II = asthenic type.
§ + + + + = gland restored to normal size.
+ + + + = almost normal size.
+ + = distinct improvement.
+ = slight improvement.
± = no improvement.

was controlled with thiouracil, the bismuth therapy was then followed by a reduction of the size of the gland to normality in two cases. It failed under similar conditions in one other case.

The patients in this series had moderately enlarged thyroids which, except in one instance, did not produce local distress. Most of the cases were considered to be simple goiter, although some had nodular enlargement and in the latter part of the series some patients with hyperfunction were also treated.

Therapy: The bismuth salts commonly used in the treatment of syphilis were employed, the subsalicylate and heptadiencarbonate being the most frequently used.² The patients were given 1 or 2 injections weekly, with a total of 20 injections in each series. There were intermissions of from two to four weeks between series. The injections were made into the gluteal region, and routine precautions against toxic reactions were taken, the gums and local area being inspected carefully and frequently.

Although no measurements of the thyroid were made, the enlargement was carefully estimated, and only when a striking decrease in the size of the gland was evident was a result set down as positive.

RESULTS

The results are listed in Table 1, which is for the most part self-explanatory.

In the following paragraphs are mentioned some of the salient features of the various cases:

There were 10 cases of diffuse simple goiter. The only males were *No. 1* and *No. 15*. The former had been treated with arsenic and bismuth for a syphilitic chancre but he had never shown positive findings in the blood. Two years after the treatment was discontinued his thyroid remained normal in size.

Case No. 3 showed hyperthyroidism with a very effective reaction to bismuth.

Case No. 4 showed very rapid improvement, the gland reaching normal size after 15 injections.

Case No. 5 was one of syphilis in which a goiter developed after anti-luetic therapy; she also had hypertension.

Case No. 9. The patient failed to improve with bismuth; she ultimately developed tuberculosis after having had a severe bout of hyperthyroidism which improved with thiouracil.

² Bismuth subsalicylate (*Stabisol*, Squibb): Bi., 0.13 Gm. \times cc. (1 cc. once a week or $\frac{1}{2}$ cc. twice a week).

Bismuth heptadiencarbonate (*Medobis*, Chinoïn): Bi., 0.045 Gm. $\times \frac{1}{2}$ cc. ($\frac{1}{2}$ cc. twice a week).

function. The bismuth therapy was given in series of injections in accordance with the common practice in the treatment of syphilis, and was particularly successful when two or more series of injections were given. When hyperthyroidism was present, the therapy did not appear to alter the state of hyperfunction, but when this condition was controlled prior to the bismuth therapy, this treatment appeared then to reduce the gland to normal size. Fifty per cent of the cases of simple or nodular goiter showed great improvement; 20 per cent showed slight improvement and 30 per cent failed to improve.



Case No. 10 was one in which there was no improvement with two series of treatment. The patient is now in the course of the third treatment. She shows an anxiety neurosis and a mild hyperostosis frontalis interna.

The next 6 cases (*Nos. 11-16*) are of nodular goiter.

Case No. 12 is noteworthy because of the success of bismuth therapy after the failure of iodine.

Case No. 13. The patient showed improvement after 40 injections. Treatment was then discontinued and during the following ten months her goiter showed renewed growth. She later developed hyperthyroidism.

Case No. 14 was one in which the goiter was associated with discomfort on swallowing. Following two series of treatments there was some decrease in size sufficient to cause the discomfort to disappear.

Cases No. 17 and 18 showed failure of bismuth therapy in Graves' disease.

DISCUSSION

There were good results in about 70 per cent of the cases treated and in 50 per cent of the cases the results were excellent; in some it was successful even when iodine had failed. Thus, of the 10 patients with diffuse goiter, 5 showed great improvement, 2 showed slight improvement, and treatment failed in 3 instances.

Among the 6 cases of nodular goiter, 3 showed great improvement, 1 improved only slightly, and 2 failed to improve.

In the 16 cases treated, 50 per cent showed great improvement, 19 per cent slight improvement, and 31 per cent failed to improve.

The duration of the treatment appeared to be important, for although 1 case was restored to normal before 15 injections were given, in the majority of the cases two series of treatments were necessary. The only 2 patients who received three series of injections showed great improvement. The following table indicates the dispersion of the cases with respect to the number of series of treatments:

Improvement according to the number of series of injections

	<i>One series</i>			<i>Two series</i>			<i>Three series</i>		
Improvement:	Great	Slight	None	Great	Slight	None	Great	Slight	None
No. of cases:	1	1	2	5	2	3	2	0	0

SUMMARY

Bismuth salts, the subsalicylate and the heptadiencarbonate such as are used in the therapy of syphilis, have been applied to the treatment of both diffuse and nodular goiter. In these cases the metallic ion seems to act upon the structure of the gland to reduce its size. It does not appear to alter its

3. Studies on an Anti-Diuretic, Non Chloruretic Substance Extracted from Urines of Normal and Cirrhotic Subjects. By Elaine P. Ralli, Stephen Leslie (by invitation), George H. Stueck, Jr. (by invitation) Mary E. Dumm and Bertram Laken (by invitation).

4. A Method for the Assay of Prolactin in Human Urine. By Richard L. Coppedge (by invitation) and Albert Segaloff.

5. Thyrotrophic and Thyroid Hormone Assay of Normal and Pathologic Human Sera in the Stasis Tadpole. By S. A. D'Angelo, A. S. Gordon, K. E. Paschkis and A. Cantarow.

6. Estimation of Urinary Gonadotrophin of the Non-pregnant Human by the Mouse Uterine Weight and Ovarian Hyperemia Responses. By Charles W. Lloyd, Muriel Morley (by invitation), Kathryn Morrow (by invitation), Julia Lobotsky (by invitation) and Edward C. Hughes (by invitation).

7. Further Studies of Antigonadotrophin Formation in Man. By James H. Leathem and A. E. Rakoff.

8. The Evaluation of the Use of Anterior Pituitary Extract in the Treatment of Pituitary Dwarfism. By Joseph C. Edwards, Cecil M. Charles (by invitation) and Cyril M. MacBryde.

9. On the Inability of Adrenocorticotrophic Hormone or Epinephrine to Deplete the Ascorbic Acid of the Chick Adrenal. By Norman F. Boas (by invitation) and Joseph W. Jailer.

10. Regulation of Pituitary Adrenocorticotrophic Activity by Adrenal Cortical Hormones. By Chi-Ping Cheng (introduced by George Sayers).

11. Adequacy of Pituitary Adrenocorticotrophic Function in Nutritional Deficiencies. By George Sayers.

12. Effects of Prolonged Adrenal Cortical Stimulation upon Free and Esterified Serum Cholesterol in Normal Men. By Jerome W. Conn and William C. Vogel (by invitation).

13. Possible Involvement of the Adrenal Cortex and Thyroid in Mobilization of Fat to the Liver. By Louis Levin.

FRIDAY, JUNE 3, 1949

2:00 p.m.—J. S. L. Browne, presiding

14. Stimulation of Nitrogen by Adrenal Cortical Extract during Insulin Hypoglycemia. By Frank L. Engel.

15. Renal Function in Normal and Adrenalectomized Rats following Saline or Adrenal Steroid Administration. By W. R. Boss (by invitation) James H. Birine and Robert Gaunt.

16. Adrenal Cortical Hormone in Blood. By K. E. Paschkis, A. Cantarow and D. Boyle (by invitation).

17. Urinary Corticoids. By Eleanor H. Venning, M. P. Ripstein (by invitation) and V. E. Kazmin (by invitation).

18. Studies on the Interrelationship of Adrenal and Thyroid Function. By Robert S. Reiss, Peter H. Forsham (by invitation) and George W. Thorn.

19. Clinical and Metabolic Changes in Addison's Disease Following the Administration of Compound E Acetate (11-dehydro, 17-hydroxy-corticosterone acetate). By P. H. Forsham (by invitation), L. L. Bennett (by invitation), M. Roche (by invitation), R. S. Reiss, A. Slessor (by invitation), E. B. Flink (by invitation) and G. W. Thorn.

20. Effect of a Single Dose of Desoxycorticosterone Acetate on Electrolyte Metabo-

The 1949 Meeting of the Association for the Study of Internal Secretions

FRIDAY AND SATURDAY, JUNE 3 AND 4

Headquarters: Chalfonte-Haddon Hall, Atlantic City, New Jersey.

Registration: Everyone attending the meetings is requested to register. A fee of \$1.00 will be charged non-members of the Association. Membership cards should be presented when registering.

The Scientific Sessions: The Scientific sessions will be held in the Viking Room of the Haddon Hall Hotel and programs will begin promptly on schedule. Papers presented at all meetings are planned for ten minutes, unless otherwise noted, and owing to the heavy schedule must be kept within this limit. Manuscripts of all papers should be submitted to the presiding officer or Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Association will be held on Friday evening, June 3, at 7:30 o'clock in the Rutland Room of the Haddon Hall, preceded by cocktails at 6:30 o'clock in the West Room. Secure tickets at time of registration.

Council Meetings: There will be a meeting of the Council on Thursday afternoon, June 2, at 2:00 o'clock, and a luncheon meeting on Friday, June 3.

Business Meeting: The Annual Business Meeting of the Association and Election of Officers will be held at 4:30 p.m., June 4, in the Viking Room of the Haddon Hall.

Local Arrangements: Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

PROGRAM

FRIDAY, JUNE 3, 1949

9:15 a.m.—C. N. H. Long, presiding

1. Piperido-methyl-benzodioxane (933-F): Some Pharmacological and Experimental Observations. By Evan Calkins (by invitation), George W. Dana (by invitation), J. C. Seed (by invitation) and John Eager Howard.
2. Nor-Epinephrine in Adrenal Medulla. By Marcel Goldenberg and Mogens Faber (introduced by R. F. Loeb).

37. Hormonal Factors Producing the Gametokinetic Response in the Male Frog (*Rana Pipiens*). By Robert B. Greenblatt, Sarah Clark (by invitation) and R. M. West (by invitation).

38. Action of Estrogens on Release of Luteinizing Hormone in Menopausal Women. By Arthur A. Hellbaum, J. W. Funnell (by invitation) and E. C. Keaty (by invitation).

39. The Hormonal Pattern in Pseudocyesis. By A. E. Rakoff and Paul H. Fried (by invitation).

40. Management of Threatened Abortion in the Human with Large Doses of Prethylstilbestrol. By A. B. Abarbanel.

SATURDAY, JUNE 4, 1949

2:00 p.m.—C. H. Best, presiding

Joint Meeting with The American Diabetes Association

41. A Hyperglycemic Factor Extracted from the Pancreas. 10 mins. By I. J. Pincus (introduced by A. E. Rakoff).

42. Studies in Carbohydrate Metabolism in Decerebrate Rats. 10 mins. By Evelyn Anderson and Webb Haymaker (by invitation).

43. Factors Affecting the Volume of the Islands of Langerhans. 15 mins. By R. E. Haist, Margaret Evans and B. Kinash (introduced by C. H. Best).

44. Studies on the Serum Potassium in Diabetic Acidosis. 15 mins. By Carl S. Nadler, Samuel Bellett and Mary Lanning (introduced by C. H. Best).

45. Pyruvic and Citric Acid Metabolism. 15 mins. By Max Miller and Ernest Bueding (introduced by C. H. Best).

46. Changes in Inorganic Serum Phosphorus during the Intravenous Glucose Tolerance Test as an Adjunct to the Diagnosis of Early Diabetes Mellitus. 15 mins. By Peter H. Forsham (by invitation), Marcel Roche (by invitation) and G. W. Thorn.

47. The Metabolism of Glucose and Galactose when Administered Simultaneously to Man. 10 mins. By G. C. Walsh (by invitation), M. M. Hoffman, H. T. McAlpine (by invitation) and E. H. Mason (by invitation).

48. Studies in Fat Metabolism. 1. Steroid Hormonal Effects Upon Blood Ketones and Other Intermediate Products of Fat and Protein Catabolism. 10 mins. By Laurance W. Kinsell, Sheldon Margen (by invitation), George D. Michaels (by invitation), Betty T. Signorotti (by invitation) and David P. McCallie (by invitation).

49. The Urinary Excretion of Corticosteroids in Diabetic Acidosis. 10 mins. By Janet W. McArthur, Randall G. Sprague and Harold L. Mason.

50. Steroid Diabetes Associated with Cushing's Syndrome and Excretion of 17-Hydroxycorticosterone (Compound F) in Urine; Metabolic Studies. 10 mins. By Randall G. Sprague, Alvin B. Hayles (by invitation), Harold L. Mason, Marschelle H. Power (by invitation) and Warren A. Bennett (by invitation).

51. Behavior of Electrolytes During Treatment of Diabetic Keto-Acidosis. 10 mins. By Jonas Weissberg, (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Dreker.

TO BE READ BY TITLE

52. Correlation of Vaginal Smears and Endometrial Biopsies in Normal Cycles and in Gynecic Disorders. By H. E. Nieburgs, Robert B. Greenblatt and S. Bamford (by invitation).

53. The Effect of Pteroylglutamic Acid Antagonists on the Response of the Reproductive Accessories of C57 Male Mice to Testosterone. By E. D. Goldsmith, H. M. Black (by invitation) and R. F. Nigrelli (by invitation).

lism. By Paul Fourman (by invitation), Edwin J. Kepler, Edward C. Reifstein, Jr. and Eleanor F. Dempsey (by invitation).

21. Changes in Urinary Steroids Produced by Sodium Deprivation and by Desoxycorticosterone Acetate Administration. By William H. Daughaday (by invitation) and Cyril M. MacBryde.

22. The Evaluation of Adrenocortical Function by Ascertaining the Response to a Single Injection of Adrenocorticotrophin. By H. W. McIntosh (by invitation), B. Singer (by invitation) and M. M. Hoffman.

23. The Level of Circulating Eosinophils as an Indicator of Adrenal Cortical Adequacy Following Major Surgery. By Marcel Roche (by invitation), A. Gorman Hills (by invitation) and George W. Thorn.

24. Is the Protein Metabolic Abnormality of Cushing's Syndrome Catabolic or Anti-Anabolic? By Sheldon Margen (by invitation), Laurence W. Kinsell, Erin K. Flanagan (by invitation), Lila E. Suiter (by invitation) and Elliot Rapaport (by invitation).

25. Hypokaliemic Alkalosis in Cushing's Syndrome. Observations on the Effects of Potassium Chloride and Testosterone Propionate Therapy. By Robert Teabeaut (by invitation), Frank L. Engel and Haywood M. Taylor (by invitation).

26. The Mechanism of Action of Testosterone in the Therapy of Cushing's Syndrome. By Frederick C. Bartter (by invitation), Anne P. Forbes, William M. Jefferies, Evelyn L. Carroll (by invitation) and Fuller Albright.

SATURDAY, JUNE 4, 1949

9:00 a.m.—E. A. Doisy, presiding

27. Metabolism and Distribution of Thiourea in the Rat as measured with Radioactive Sulfur. By John Schulman, Jr. and Richard P. Keating (introduced by Rulon W. Rawson).

28. The Tracer Technique with Radioiodine I^{131} as a Potential Substitute for the Basal Metabolic Rate Determination in Routine Clinical Practice. By Sidney C. Werner.

29. The Distribution and Metabolism of Circulating Testosterone. By C. D. West and L. T. Samuels.

30. Pseudo-hypoparathyroidism: A Report of Two New Cases with Special Reference to the Epiphyseal Changes. By Harold Elrick (by invitation), Frederic C. Bartter (by invitation), Adney Sutphin (by invitation) and Fuller Albright.

31. Quantitative Measurements of the Growth of Axillary Hair as an Index of the Endocrine Status. By James B. Hamilton.

32. The Effects of Testosterone Propionate on the Peripheral Blood and Bone Marrow of Women with Advanced Carcinoma of the Breast. By Timothy R. Talbot, Jr. (by invitation) and George C. Escher.

33. Effects of Small Doses of Testosterone Propionate on Spermatogenesis. By Cleve Beller (by invitation) and Henry H. Turner.

34. Endocrine Factors in Gout: The Significance of Differences in Childhood and Adult Urate Metabolism. By William Q. Wolfson, David Krevsky, Rachmiel Levine (by invitation), Kinu Kadota (by invitation) and Clarence Cohn.

35. The Effect of Castration, of Unilateral Castration and of Pregnancy in Unilaterally Castrate Rats on the Ovary Transplanted into the Spleen. By Gerson R. Biskind and Morton S. Biskind.

36. The Occurrence of Conjugated Sulfates of Estrogens in Human Pregnancy Urine. By Herman Cohen (by invitation) and Robert W. Bates.

The 1949 Annual Meeting of the American Diabetes Association

CHALFONTE-HADDON HALL,

ATLANTIC CITY, N. J.

SATURDAY AFTERNOON, JUNE 4; Joint Meeting with the Association for
the Study of Internal Secretions.

SUNDAY MORNING AND AFTERNOON, JUNE 5.

BANQUET, SATURDAY NIGHT.

Please send reservations for the banquet now to this office. Wives of members are welcome. Dinner subscriptions—\$6.00—*Payable when you register at the meeting.*

Postgraduate Course in Endocrinology, Including Diabetes

THE MEDICAL CENTER, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL
Parnassus and Third Avenues, San Francisco 22, California

JUNE 20 THROUGH 24, 1949

TOLAND HALL, UNIVERSITY OF CALIFORNIA HOSPITAL

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Medicine, University of California Medical School.

Chairman of Program: Roberto F. Escamilla, M.D., Associate Clinical
Professor of Medicine, University of California Medical School.

Officers of Instruction:

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This course is open only to graduates of medical schools approved by the Council on Medical Education and Hospitals of the American Medical Association.

54. Synthesis of Testosterone from Androstenedione-3, 17 by Testis Tissue. By Leo T. Samuels, Blaine H. Levedahl (by invitation), M. L. Helmreich (by invitation) and M. M. Pottner (by invitation).
55. The Role of the Adrenal Cortex in Some Somato-Sexual Aberrations in Infants and Children. By M. M. Melicow.
56. Effects of Compound E on Blood Ketone Bodies. By Leslie L. Bennett (by invitation), Alexander Slessor (by invitation) and George W. Thorn.
57. The Effect of Dietary Protein on the Ability of the Liver to Inactivate Estradiol in the Rat. By Joseph W. Jailer.
58. Pregnanediol Excretion in Cases of Blighted Ovum. By A. B. Abarbanel, Robert Hoyt (by invitation) and M. G. Levine (by invitation).
59. A Mechanism of Potassium Deficiency in Alkalosis. By Charles H. Burnett, Belton A. Burrows (by invitation) and Robert R. Commons (by invitation).
60. Effects of Hemopoietic Agents on Blood Formation in Hypophysectomized Rats. By Robert Gerstner (by invitation) and Albert S. Gordon.
61. The Relative Effectiveness of Desoxycorticosterone Acetate in Oil Solution and in Pellets Diluted with Cholesterol. By Albert Segaloff.
62. Adrenal Cortex Activity in Essential Hypertension. By Louis Tobian, Jr. and Harold Joseph (introduced by Carl A. Bunde).
63. The White Blood Cell Response of Rats to Adrenalectomy, Stress, and Pantothenic Acid. By Mary E. Dumm, Paul Roth (by invitation), Paul Ovando (by invitation) and Elaine P. Ralli.
64. Role of Emotional Stress in the Survival of Adrenalectomized Rats given Replacement Therapy. By Miguel R. Covian (introduced by Curt Richter).
65. The Androgenic Activity of New Esters of Testosterone. By A. J. Bergmann and Lloyd C. Miller (introduced by John S. L. Browne).
66. Effect of Androgen and Growth Hormone on the Rat's Os Penis. By Wm. R. Lyons, Edward Abernethy (by invitation) and Mark Grooper (by invitation).
67. The "Thiocyanate Space" and "Iodide Space" in the Thyroid Gland. By J. F. McClendon, William C. Foster (by invitation) and Emerson Reed (by invitation).
68. A Comparison of the 17-Ketosteroid Excretion of Cases of Cushing's Syndrome Due to Adrenal Tumor with Those Due to Hyperplasia (Hyperfunction). By Anne P. Forbes, Evelyn L. Carroll (by invitation) and Mary L. Wheeler (by invitation).
69. Sex Hormones and Staphylococcus Infections. By Manuel Villaverde.
70. The Problem of Allergy to Steroid Hormones. By George P. Heckel.
71. Intravenous Estrogen in Menometrorrhagia in the Human. By A. R. Abarbanel.
72. The Incidence of Cancer in Endocrine Case Histories. By J. K. Fancher and Jean Brooks (by invitation).
73. Renal Clearances in Patients with Cirrhosis of the Liver, with and without Ascites. By Stephen H. Leslie, Barbara Johnson (by invitation) and Elaine P. Ralli.
74. Porphyria Simulating Anorexia Nervosa. By Bernard A. Watson.
75. Hemosedimentation Test in Obesity. Aulo Pinto Viégas.
76. The Effects of Vitamin B, Thyroid, and Adrenal Alterations on the Amino Acid Oxidase Activity of Rat Liver and Kidney. By Samuel R. Tipton and Frances M. Colvin (by invitation).
77. The Problem of Endemic Goiter in Yunnan Province. By Isidor Greenwald.

Books Received

THE following list acknowledges receipt of books which it has not been possible for us to review as yet:

- La Citologia Vaginal Humana en Condiciones Normales y Patalógicas.* By INÉS L. C. DE ALLENDE, Doctora en Medicina, Jefe de la Sección Endocrinología del Instituto de Investigación Médica, Córdoba, Argentina, and OSCAR ORÍAS, Doctor en Medicina, Director del Instituto de Investigación Médica, Córdoba, Argentina. Prólogo del Prof. Dr. BERNARDO A. HOUSSAY. 313 pages. 105 illustrations (53 in color). 1947. El Ateneo, Buenos Aires, Argentina.
- Clinical Endocrinology and Constitutional Medicine.* By A. P. CAWADIAS, O.B.E., M.D., F.R.C.P., Endocrinologist to the Order of St. John Clinic. 368 pages. 14 illustrations. 1947. Frederick Muller, Ltd., London, England. Price 42'-net.
- Crystalline Enzymes.* 2nd Ed. revised and enlarged. By JOHN H. NORTHROP, Member, The Rockefeller Institute for Medical Research, Princeton, New Jersey, and sharer of the Nobel Prize in Chemistry for 1946; MOSES KUNITZ, Associate Member, The Rockefeller Institute for Medical Research, and ROGER M. HERRIOTT, Associate, The Rockefeller Institute for Medical Research. 352 pages. 105 illustrations. 1948. Columbia University Press, New York. Price \$7.50.
- Los Desordenes de la Menstruacion y su Tratamiento: Base Experimental de la Interrelación Prehipófiso-ovárica.* By DR. EMILIO COLOMBO. 327 pages. 159 figures. 1949. El Ateneo, Buenos Aires.
- Diabetes and its Treatment.* By JOSEPH H. BARACH, M.D., F.A.C.P., Associate Professor of Medicine, University of Pittsburgh; Senior Medical Staff, Presbyterian Hospital; Medical Director, Outpatient Department of the Medical Center Hospitals; School of Medicine, University of Pittsburgh. 326 pages. 73 illustrations. 1949. Oxford University Press, New York. Price \$10.00.
- The Diabetic's Handbook.* By ANTHONY SINDONI, JR., M.D., Chief of the Department of Metabolism, Philadelphia General and St. Joseph Hospitals; Chairman of the Advisory Committee on Diabetes to the Director of the Department of Public Health, Philadelphia; Chief of the Diseases of the Metabolism, St. Francis Hospital, Wilmington, Delaware. 194 pages. 7 illustrations. 1948. The Ronald Press Company, New York. Price, \$3.00.
- Diagnosis and Treatment of Menstrual Disorders and Sterility.* 2nd Ed. revised and enlarged. By CHARLES MAZER, M.D., F.A.C.S., Assistant Professor of Gynecology and Obstetrics, Graduate School of Medicine, University of Pennsylvania; Gynecologist to the Mount Sinai Hospital, Philadelphia, and S. LEON ISRAEL, M.D., F.A.C.S., Instructor in Gynecology and Obstetrics, School of Medicine, University of Pennsylvania; Associate Gynecologist to the Mount Sinai Hospital, Philadelphia. 570 pages. 133 illustrations. 1946. Paul B. Hoeber, Inc., New York. Price, \$7.50.
- Diagnosis in Sterility.* 1st Ed., 2nd printing. Proceedings of the Conference on Sterility, sponsored by the National Committee on Material Health. Edited by EARL T. ENGLE, College of Physicians and Surgeons, New York. 237 pages. 30 illustrations. 1947. Charles C Thomas Publisher, Springfield, Illinois. Price \$5.50.
- Diagnostic Hormonal et Traitements Hormonaux en Gynécologie.* By CLAUDE BÈCLÈRE. Préface by Professeur H. SIMONNET. 373 pages. 1946. Masson et C^{ie}, Paris. Price 525 fr.

The fee for the course is \$50.00, payable at the time of enrollment, either by check or money order made payable to THE REGENTS OF THE UNIVERSITY OF CALIFORNIA. Please indicate the course for which application is being made.

University Extension reserves the right to cancel this course, in which case all fees will be refunded.

Application is to be made to: Stacy R. Mettier, M.D., Head of Postgraduate Instruction, Medical Extension, University of California Medical Center, San Francisco 22, California. Telephone: MONTrose 4-3600, local 255.

VETERANS

Those applying for educational benefits under federal or state statutes will present, at the time of registration, their Certificates of Eligibility and Entitlement which will be accepted in lieu of the fee. Veterans who previously have filed their Certificates with University Extension and have not used their educational benefits elsewhere in the interim will likewise be admitted without fee, provided they have sufficient credit left on their Certificates. Application for the Certificate of Eligibility and Entitlement may be made through local offices of the Veterans Administration, and *must be dated prior to the beginning of the course.*

- The Modern Treatment of Diabetes Mellitus, Including Practical Procedures and Precautionary Measures.* By WILLIAM S. COLLENS, B.S., M.D., Chief of the Diabetic Clinic, and of the Clinic for Peripheral Vascular Diseases and Associate Visiting Physician, Israel Zion Hospital, Brooklyn; Associate Visiting Physician, Greenpoint Hospital, Brooklyn; Attending Metabolist, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn; and LOUIS C. BOAS, A.B., M.D., Assistant in the Diabetic Clinic and in the Clinic for Peripheral Vascular Diseases, Israel Zion Hospital, Brooklyn; Chief of the Diabetic Clinic and Assistant Visiting Physician, Greenpoint Hospital, Brooklyn; Associate in Department of Metabolism, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, New York. 514 pages. 193 illustrations. 1946. Charles C Thomas Publisher, Springfield, Illinois. Price \$8.50.
- Mongolism and Cretinism.* By CLEMENS E. BENDA, M.D., Director Wallace Research Laboratory for the Study of Mental Deficiency, Wrentham, Massachusetts; Instructor in Neuropathology, Harvard Medical School; Assistant in Psychiatry, Massachusetts General Hospital; Lecturer, Postgraduate Seminar, Massachusetts Department of Mental Health. 310 pages. 103 illustrations. 1946. Grune & Stratton, Inc., New York. Price \$6.50.
- Natural Products Related to Phenanthrene.* 3rd Ed. By LOUIS F. FIESER and MARY FIESER, Department of Chemistry, Harvard University. 704 pages. 1949. Reinhold Publishing Corporation, New York. Price \$10.00.
- Office Endocrinology.* 3rd Ed. Revised and enlarged. By ROBERT B. GREENBLATT, B.A., M.D., C.M., Professor of Endocrinology, University of Georgia School of Medicine; Director, Sex Endocrine Clinic, University Hospital, Augusta, Georgia. With a foreword by G. LOMBARD KELLY, M.D., Dean, University of Georgia School of Medicine. 306 pages. 71 figures. 1947. Charles C Thomas Publisher, Springfield, Illinois. Price \$4.75.
- Pathology and Surgery of Thyroid Disease.* By JOSEPH L. DECOURCY, M.D., Senior Surgeon, Good Samaritan Hospital; Director, DeCourcy Clinic, Cleveland, Ohio, and CORNELIUS B. DECOURCY, M.D., Member DeCourcy Clinic Surgical Staff, Cincinnati, Ohio. 476 pages. 94 illustrations (4 in color). 1949. Charles C Thomas Publisher, Springfield, Illinois.
- Practical Aspects of Thyroid Disease.* By GEORGE CRILE, JR., M.D., F.A.C.S., Department of Surgery, Cleveland Clinic. 354 pages. 101 illustrations. 1949. W. B. Saunders Company, Philadelphia.
- Practical Office Gynecology.* By KARL JOHN KARNAKY, M.D., Assistant Professor of Clinical Gynecology, Baylor University College of Medicine; Gynecologist to Jefferson Davis Hospital, Houston, Texas. 261 pages. 113 figures showing 238 illustrations; 29 figures are in color, showing 73 illustrations. 1947. Charles C Thomas Publisher, Springfield, Illinois. Price \$7.50.
- The Practice of Endocrinology.* Edited by RAYMOND GREENE, M.A., D. M., M.R.C.P. with contributions by A. C. CROOKE, M.A., M.D., Clinical Endocrinologist Birmingham United Hospital, the Birmingham and Midland Hospitals for Women, and the Children's Hospital, Birmingham, England; DONALD HUNTER, M.D., F.R.C.P., London Hospital; R. D. LAWRENCE, M.A., M.D., Physician in charge of the Diabetic Department of King's College Hospital, London; J. M. ROBSON, M.D., D.Sc., F.R.S.E., reader in Pharmacology at Guy's Hospital Medical School, London; F. F. RUNDLE, M.D., F.R.C.S., Assistant Surgeon and Assistant Director of the Surgical Professorial Unit at St. Bartholomew's Hospital, London; Rockefeller Travelling Fellow; formerly Hunterian Professor, Royal College of Surgeons, London; and P. H. SANDIFIER, M.R.C.P., of the Maida Vale Hospital for Nervous Diseases; Neurological Physician Royal National Orthopaedic Hospital, London. 366 pages. 19 figures and 53 plates (1 colored). 1948. Eyre & Spottiswoode, London, England. Price 52s. 6d.

- Diseases of the Adrenals.* 2nd Ed. By LOUIS J. SOFFER, M.D., Associate Attending Physician, The Mount Sinai Hospital, New York City; Assistant Clinical Professor of Medicine, Columbia University, New York. 320 pages. 45 illustrations (3 color plates). 1948. Lea & Febiger, Philadelphia. Price \$6.50.
- Endocrine Function of the Hypophysis.* By HARRY B. FRIEDGOOD, M.D., Assistant Clinical Professor of Medicine, University of Southern California and Senior Attending Physician, Los Angeles County Hospital, Los Angeles, California. Edited by HENRY A. CHRISTIAN, A.M., M.D., LL.D., Sc.D(Hon.), F.A.C.P., Hon. F.R.C.P. (Can.), Hersey Professor of the Theory and Practice of Physics, Emeritus, Harvard University; Clinical Professor of Medicine, Tufts Medical Physician-in-Chief, Emeritus, Peter Bent Brigham Hospital; Visiting Physician, Beth Israel Hospital, Boston, Massachusetts. (Reprinted from Oxford Loose-Leaf Medicine, with the same page numbers as in that work). 240 pages. 36 illustrations. 1946. Oxford University Press, New York.
- Endogeneous Endocrinotherapy Including The Causal Cure of Cancer.* (Compendium) By DR. JULES SAMUELS, Specialist for endogeneous endocrinotherapy, Amsterdam. 539 pages. 30 illustrations. 1947. Holdert & Co., Amsterdam.
- Essai de Physiopathologie Thyro-Hypophysaire.* By Dr. JACQUES MAHAUX, Agrégé de l'enseignement supérieur, Adjoint de la Clinique médicale universitaire (Belgique). Préface du Professeur E. J. BIGWOOD. 268 pages. 29 figures. 1947. Masson et C^{ie}, Paris. Price 530 fr.
- Essentials of Endocrinology.* 2nd Ed. revised and enlarged. By ARTHUR GROLLMAN, Ph.D., M.D., F.A.C.P. Professor of Medicine, The Southwestern Medical College; Attending Physician and Consultant in Endocrinology. The Parkland Hospital, Dallas, Texas. 644 pages. 132 illustrations. 1947. J. B. Lippincott Company, Philadelphia.
- Essentials of Gynecologic Endocrinology.* By GARDNER M. RILEY, Ph.D., Assistant Professor of Obstetrics and Gynecology, University of Michigan Medical School. 205 pages. 35 figures and 1 color plate. 1948. Caduceus Press, Ann Arbor, Michigan.
- Experimental Immunochemistry.* By ELVIN A. KABAT, Ph.D., Associate Professor of Bacteriology, College of Physicians and Surgeons, Columbia University and the Neurological Institute, New York; and MANFRED M. MAYER, Ph.D., Associate Professor of Bacteriology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland. With a foreword by MICHAEL HEIDELBERGER, Ph.D., Professor of Biochemistry, College of Physicians and Surgeons, Columbia University, and Chemist to the Presbyterian Hospital, New York. 575 pages. 88 illustrations. 1948. Charles C Thomas Publisher, Springfield, Illinois. Price \$8.75.
- Food and Facts for the Diabetic.* By JOSEPH H. BARACH, M.D., F.A.C.P., Associate Professor of Medicine, School of Medicine, University of Pittsburgh. 111 pages. 16 figures. Oxford University Press, New York. 1949. Price \$4.00.
- General Endocrinology.* By C. DONNELL TURNER, Ph.D., Associate Professor of Zoology at Northwestern University. 604 pages. 164 illustrations. 1948. W. B. Saunders Company, Philadelphia.
- Hormones and Behavior—A Survey of Interrelationships Between Endocrine Secretions and Patterns of Overt Response.* By FRANK A. BEACH, Professor of Psychology, Yale University. With a foreword by EARL T. ENGLE, M.D., College of Physicians and Surgeons, Columbia University, New York. 368 pages. 1948. Paul B. Hoeber, Inc., New York and London. Price \$6.50.
- L'Hyperfolliculine—Étude Clinique, Anatomo-Pathologique et Thérapeutique.* By MAX WALLET. Preface by L. de GENNES. 354 pages. 25 figures. 1946. Masson et C^{ie}, Paris. Price 480 fr.

Abstracts of

CURRENT ENDOCRINE LITERATURE

Editor: ROY HERTZ. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

ADRENALS

GREEN, D. M.: Mechanisms of desoxycorticosterone action. I. Relation of fluid intake to blood pressure, *J. Lab. & Clin. Med.* 33: 853-859, 1948.

Since the results of preliminary investigations suggested that desoxycorticosterone (DCA) may possess the ability to elevate mean pressure without production of secondary cardiac slowing or pulse pressure increase, studies were made of the mechanisms by which this drug affects the height of the blood pressure level. Implantation of a single 20 mg. pellet of DCA in young rats maintained on isotonic saline solution was followed immediately by a rise in fluid intake, later by a secondary regression of intake values toward control levels and the reciprocal development of hypertension. The degree of this hypertension appeared to be in proportion to the dosage of the drug, the maximal rise in fluid intake, and the subsequent rate of decline in intake. Adrenalectomy apparently did not sensitize the test animals to the actions of DCA. The author suggests that the hypertension induced by overdosage of DCA may not represent a direct action, but may be a compensatory mechanism for overcoming distortions in fluid and electrolyte balance produced by the drug.—T.H.McG.

LA DUE, J. S.; MURISON, P. J., and PACK, G. T.: The use of tetraethylammonium bromide as a diagnostic test for pheochromocytoma, *Ann. Int. Med.* 29: 914-921, 1948.

The responses of a patient with pheochromocytoma to the parenteral administration of histamine diphosphate and of tetraethylammonium bromide are presented. Both agents induced a paroxysm of severe hypertension. However, that following the administration of histamine was uncontrollable, whereas the level and duration of blood pressure rise after the administration of tetraethylammonium bromide could be controlled by a change in the patient's posture.—J.M.

MASON, H. L., and SPRAGUE, R. G.: Isolation of 17-hydroxycorticosterone from the urine in a case of Cushing's syndrome associated with severe diabetes mellitus, *J. Biol. Chem.* 175: 451-56, 1948.

In this case of Cushing's syndrome, associated with bilateral hyperplasia of the adrenal cortex, 191 mg. of 17-hydroxycorticosterone was isolated from the urine collected during a 25-day period. The total corticosteroids excreted, as measured by a chemical procedure, was 14 to 19 mg. per day. Isolation of this adrenal cortical hormone, which is the most active one known with respect to carbohydrate metabolism, supports the view that at least some of the manifestations of Cushing's syndrome are primarily the result of an overproduction of those cortical hormones which are active in carbohydrate metabolism.

- Pregnancy Diagnosis Tests: A Review.* By ALFRED T. COWIE, B.Sc., M.R.C.V.S., Ph.D., National Institute for Research in Dairying, University of Reading. 283 pages. 1948, Joint Publication No. 13 Commonwealth Agriculture Bureaux, Penglais, Aberystwyth, Great Britain. Price 15s.
- Psychodynamics and the Allergic Patient.* By HAROLD A. ABRAMSON, M.D., F.A., F.A.C.A., Associate Physician for Allergy, The Mount Sinai Hospital, New York, New York; Consulting Physician for Allergy, Sea View Hospital, Staten Island, New York; Assistant Professor of Physiology, Columbia University, New York. 81 pages. 1948. The Bruce Publishing Company, St. Paul and Minneapolis.
- Recent Advances in Endocrinology.* 6th Ed. By A. T. CAMERON, C.M.G., M.A., D.Sc. (Edin.) F.R.I.C., F.R.S.C., Professor of Biochemistry, University of Manitoba, and Biochemist at the Winnipeg General Hospital, Winnipeg, Canada. 443 pages. 74 illustrations. 1947. The Blakiston Company, Philadelphia and Toronto. Price \$6.00.
- On the Relation Between the Thyroid Gland and Uterine Myoma.* By MAURI ROUHUNKOSKI. 83 pages. 1948. Mercatorin Kirkapaino, Helsinki.
- The Renal Origin of Hypertension.* By HARRY GOLDBLATT, M.D., C.M., Director, Institute for Medical Research, Cedars of Lebanon Hospital; Professor of Pathology, University of Southern California, Los Angeles, California. A monograph in American Lectures in Pathology, edited by PAUL R. CANNON. 126 pages. 38 illustrations. 1948. Charles C Thomas Publisher, Springfield, Illinois. Price \$2.75.
- Sexual Endocrinology of Non-Mammalian Vertebrates.* By L. H. BRETSCHNEIDER and J. J. DUYVENE DE WIT, from the laboratory for General Zoology, University of Utrecht, Netherlands. Number 11 in Monographs on the Progress of Research in Holland During the War. 146 pages. 96 illustrations. 1947. Elsevier Publishing Company, Inc., New York. Price \$2.75.
- Sterility and Impaired Fertility. Pathogenesis, Investigation and Treatment.* 2nd Ed. By CEDRIC LANE-ROBERTS, C.V.O., M.S., F.R.C.S., F.R.C.O.G., Gynaecological Surgeon Royal Northern Hospital, Consulting Obstetric Surgeon, Queen Charlotte's Hospital; ALBERT SHARMAN, M.D., Ph.D., M.R.C.O.G., Senior Assistant Surgeon, Royal Samaritan Hospital for Women, Glasgow, Assistant Lecturer in Clinical Gynaecology, University of Glasgow; KENNETH WALKER, M.A., M.B., B.C. (Cantab), F.R.C.S., F.I.C.S., Jacksonian Prizeman and Hunterian Professor, Royal College of Surgeons, Emeritus Surgeon, Royal Northern Hospital, Andrologist, Philip Hill Parenthood Clinic; B. P. WIESNER, D.Sc., Ph.D., F.R.S.E., Consulting Biologist, Royal Northern Hospital; and MARY BARTON, M.B., B.S., First Assistant to the Fertility Clinic, Royal Free Hospital, London. 400 pages. 96 illustrations. 1948. Paul B. Hoeber, Inc., New York.
- Temporary Rise in the Frequency of Thyrotoxicosis in Denmark 1941-1945.* By KURT IVERSEN. Translated from the Danish by Hans Andersen, M.D. 244 pages. 1948. Rosenkilde and Bagger, Copenhagen.
- Traité de Neuro Endocrinologie. Le Système Neuro-Endocrinien, le Complexe Hypothalamo-Hypophysaire, la Neuro-Ergonomie, et son Evolution Récente.* By GUSTAVE ROUSSY and MICHEL MOSINGER. 1106 pages. 261 figures. 1946. Masson et C^{ie}, Paris. Price 2200 fr.
- Tuberculosis of the Knee Joint.* By JOHANNES MORTENS. Translated from the Danish by AXEL ANDERSEN. 550 pages. 1948. Einar Munksgaard, Copenhagen, and K. H. Lewis & Co. Ltd., London.
- Die Weiblichen Sexualhormone in der Pharmakotherapie.* By Dr. Med. O. MÜHLBOCK, Amsterdam. Prof. Dr. Med. H. KNAUS, Graz; and Doz. Dr. Med. E. TSCHERNE, Graz. 300 pages. 43 illustrations (4 in color). 1948. Medizinischer Verlag Hans Huber, Bern. Distributed in U.S.A. and Canada by Grune & Stratton, Inc., New York.

SEVRINGHAUS, E. L.: Use of estrogens in medicine, *Ann. Int. Med.* 29: 595-600, 1948.

The physiologic actions of the estrogens and possible modes and routes of administration are briefly reviewed. The utilization of estrogenic therapy in the menopausal syndrome, dysmenorrhea, and adolescent hypoplasia of the breasts or uterus is discussed. Absolute and relative contraindications to the use of estrogens are considered.—J.M.

TYLER, E. T.; PAYNE, S., and KIRSCH, H.: Pregnenolone in male infertility, *West. J. Surg.* 56: 459-463, 1948.

Pregnenolone was administered to 25 males with varying degrees of infertility. In the dosage employed, the compound failed to stimulate any definite increase in sperm production. A questionable improvement in sperm motility was observed in a small percentage of the patients treated.—J.M.

VAN BRUGGEN, J. T.: A comparison of methods used for the hydrolysis of conjugated urinary estrogens, *J. Lab. & Clin. Med.* 33: 207-215, 1948.

Several experiments were carried out in order to investigate the various factors which affect the hydrolysis and extraction of conjugated urinary estrogens. Results confirmed previous suggestions that the use of 15 volumes per cent HCl for 10 minutes as the hydrolyzing medium does not give optimum yields of the conjugated estrogens. Nitrogen and sulfonic acid, in contrast to their effectiveness in experiments with pure estrogens, apparently offered little advantage with the combined forms. Two-hour pressure hydrolysis at 120° C. of aliquots of pooled urine, all samples first being adjusted to pH 1.0 with HCl or H₂SO₄, proved to be very effective. The hydrolysis of combined forms of estrogens in butanol gave the highest yields (148,000 units in the 2-hour fraction). Use of a pooled sample of urine appeared to nullify individual differences in urine samples.—T.HMcG.

VOGEL, M.; MCGAVACK, T. H., and MELLOW, J.: Effects of various estrogenic preparations on the vaginal mucosa, *Am. J. Obst. & Gynec.* 56: 269-280, 1948.

Fifty-five postmenopausal patients, ranging in age from 27 to 85 years, were studied. Vaginal smears were obtained prior to therapy, and at 12-hour intervals following administration of a single dose. Periods of observation ranged from 60 to 377 hours. The smears were stained by the Papanicolaou technique. Free estradiol, estradiol dipropionate, estradiol benzoate in sesame oil, peanut oil and propylene glycol, were injected in 1 to 5 milligram amounts. The response noted was as follows: 3 patients, no response; 24 patients, an alteration in type of smear without follicular reaction; and 44 patients, varying degrees of follicular response. The authors found that the degree of follicular reaction and amount of hormone injected could be directly correlated, that an inverse relation existed between age of patient and height of follicular reaction, and that the lag-time between treatment and appearance of the follicular reaction was 42 to 180 hours. The duration of follicular reaction varied from 12 to 200 plus hours, and, in general, the higher doses tended to produce the longer changes. Esters of estradiol in oily menstrua act more rapidly than either free estradiol or its esters, in propylene glycol. It is stressed that there is considerable individual variation in reaction to estrogens, and that doses of estrogen necessary for relief of climacteric symptoms are not quantitatively related to those required to produce a full estrogenic response in the vaginal epithelium.—C.D.D.

ism. The presence of severe diabetes which was refractory to insulin was analogous to the insulin-resistant hyperglycemia and glycosuria induced in rats by administration of the carbohydrate-active cortical hormones.—*H.L.M.*

SELYE, H.: The alarm reaction and the diseases of adaptation, *Ann. Int. Med.* 29: 403-415, 1948.

The author reviews the principal observations upon which the concept of "diseases of adaptation" is based, with special reference to nephrosclerosis and hypertension. The pathogenicity of mineralocorticoids produced in response to stress can apparently be modified by several metabolic factors. Diets poor in sodium and protein and treatment with acidifying salts tend to prevent experimentally-induced nephrosclerosis and hypertension. The application of these observations to clinical medicine is briefly discussed.—*J.M.*

GONADS

ABARBANEL, A. R.: Artificial reproduction of the cyclic changes in cervical mucus in human castrates: with clinical correlations, *West. J. Surg.* 56: 26-34, 1948.

By the administration of estrogens to female castrates (with a stump of cervix remaining) it was possible to duplicate the alteration in the cervical mucus characteristic of the immediate pre-ovulatory or co-ovulatory phase of the cycle. These alterations consist of a decided increase in the volume of cervical secretions, a marked decrease in viscosity and almost complete absence of polymorphonuclear leukocytes. This clear watery mucus is rapidly penetrable by relatively large numbers of sperm which remain actively motile in it for 24 to 72 hours, a longer time by far than in mucus obtained at any other time in the cycle. In control castrates progesterone alone, as well as testosterone preparations, were not observed to stimulate the flow of cervical mucus.—*J.M.*

FRIED, P. H.: The two hour pregnancy test, based on rat ovary hyperemia, *West. J. Surg.* 56: 552-555, 1948.

The rat hyperemia test was performed by the author on specimens obtained from 500 pregnant and nonpregnant patients, with an over-all accuracy of 96.8 per cent. The advantages cited for the test, as compared to the conventional methods, are its increased sensitivity and rapidity, the scarcity of false positive reactions, the increased tolerance of the rat to toxic specimens of urine and the convenience of the test and test animals. Disadvantages are the false negative reactions and the lack of a sharp positive endpoint.—*J.M.*

GUTERMAN, H. S., and SCHROEDER, M. S.: A simplified technique for the quantitative colorimetric estimation of pregnandiol in urine, *J. Lab. & Clin. Med.* 33: 356-366, 1948.

In contrast to the majority of quantitative methods for estimation of urinary pregnandiol, the colorimetric procedure described requires only small amounts of urine, manipulation is technically simple, and no special apparatus and chemicals are needed. The quantitative determination of pregnandiol in a given specimen of urine depends on its spectrophotometric absorption at 430 millimicra. The estimation of pregnandiol alone is possible with this method; androsterone does not interfere with the color reaction. Results of determinations in the menstrual cycle and in pregnancy check closely with those obtained with the longer gravimetric procedure as observed in other laboratories.—*T.H.McG.*

death to autopsy ranged from two to twenty-seven hours, with an average of 10.7 hours.

The pancreas was trimmed of excess fat by the pathologist, and a small block of tissue removed for routine microscopic study. The remainder was weighed and taken immediately to the laboratory.

The pancreatic tissue (40–200 Gm. per patient; average 82 Gm.) was ground in a household meat grinder in the refrigerator and mixed with 1.5 ml. of extracting fluid per gram. The extracting fluid consisted of 35 ml. of 1:4 dilution of concentrated H_2SO_4 per liter of 95 per cent ethanol. The pooled ground tissue was stored for several weeks in a large glass jar until 10 to 20 pancreases had been collected. The quantities given in the following section are based on 1 kilogram of pancreas.

The acid alcohol extract was separated from the grindings by filtering through gauze and pressing out as much liquid as possible. The extraction was then repeated with 1000 ml. of 70 per cent alcohol. After standing for two hours at room temperature, the solid matter was again removed by filtering through gauze. The filtrates were then combined and passed through filter paper. The final filtrate (about 2500 ml.) was transferred to a large dish, and the alcohol was evaporated by directing the stream of an electric fan over the surface. As the alcohol evaporated, the lipids precipitated out, and the aqueous layer became less colored. When the odor of alcohol was gone, at which time the volume was reduced to about $\frac{1}{4}$, the liquid fats were removed from the amber-colored aqueous layer in a separatory funnel, and the solids were removed by filtration.

Sodium chloride to a concentration of 25 per cent was added to the filtrate in a beaker, whereupon a dark green precipitate settled out. On standing for a few days in the ice box, more precipitate formed and adhered to the beaker, facilitating decantation. The supernatant fluid was poured off through a Buchner funnel, care being taken not to disturb the precipitate until most of the supernatant liquid had been poured off, in order to avoid clogging the filter paper.

Following the addition of 100 ml. of dilute HCl , most of the precipitate dissolved, forming a turbid green-brown solution containing some insoluble material. The suspension was transferred to a centrifuge tube, and 15 Gm. of NaCl was added, whereupon a precipitate reappeared. After standing overnight in the ice box, the tube was spun at 2000 rpm for 30 minutes and then decanted through a Buchner filter in order to collect a portion of the precipitate which floated. The precipitate was then dissolved in 25 ml. of dilute HCl , again producing a turbid green-brown solution, the pH of which was 1.85. Preparatory to filtration through a Seitz filter the pH was adjusted to 7.8 using $\text{N}/10$ NaOH . As the iso-electric point of insulin was passed, a heavy dark green precipitate formed and redissolved. The solution was filtered through a Buchner filter (under suction) and then through a sterile Seitz bacterial filter, through which buffered saline of pH 7.4 was first passed until no change in the pH of the saline solution occurred. Adjustment of the insulin solution to pH 7.8 and rinsing of the filter pad with buffer solution were done in order to avoid possible shift towards the iso-electric point of insulin (pH 5.35) with consequent precipitation and loss in the filter pad. The fine dark green precipitates, collected by filtration through the Buchner and Seitz filters, were discarded. Ten per cent phenol was added to make a final concentration of 0.5 per cent, and the pH was lowered to 2.5 with sulfuric acid. The solution was again hazy at this point, but cleared upon centrifugation at 1800 rpm for 30 minutes. The sediment, which was

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ON THE PREPARATION OF HUMAN INSULIN FOR EXPERIMENTAL USE*

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THE purpose of this paper is to describe our experience in the preparation of human insulin for experimental use. The method is a modification of one described by Somogyi, Doisy, and Shaffer (1) for the preparation of beef insulin.

Others have presented evidence (2) indicating that insulins from various sources, including human, are immunologically identical. Studies in this laboratory have not been in accord with this conclusion, in that a marked difference was observed between the response to human insulin and commercial beef and pork insulins in a patient with insulin-resistant diabetes (3) and also in a rabbit immunized with commercial insulin (4).

METHOD

Fifty pancreases were collected at the autopsy tables of the Boston City Hospital, Beth Israel Hospital, and Massachusetts Memorial Hospitals from patients dying with heart disease, liver disease, cerebral accidents, malignancy, renal disease, and various other causes. Cases of diabetes, tuberculosis, or severe sepsis were excluded. The age of the patients varied from 12 to 90 years, the average age being 60 years. An effort was made to obtain material as soon after death as possible without disturbing the routine of the respective departments of pathology. The elapsed time from

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whereas the pancreas from diabetic individuals contained an average of less than 40 units per pancreas or 0.4 units per gram. They extracted only sufficient amounts for assay by a method which may be more efficient than ours, but which would be cumbersome and expensive for producing insulin in any quantity. There are several factors which may account for the lower yield obtained by us (50 units per pancreas or 0.62 u/Gm.) as compared to that obtained by Scott and Fisher from their nondiabetic group (173 units per pancreas or 1.7 u/Gm.):

1. They obtained pancreases from persons who died suddenly; we obtained ours from patients dying in the hospital, generally after a considerable period of debility.

2. The average age in their group was 41.2 years; in ours it was 60.4 years.

3. Their material was slightly fresher than ours, being obtained on an average of 9.6 hours after death as against 10.7 hours. This slight difference probably did not play a significant part.

4. The pancreases obtained by them were larger than those obtained by us, weighing an average of 119.7 grams compared with 91.6 grams. Part of the difference (about 15 grams) can be ascribed to tissue removed for microscopic study by the pathologist. Some difference in weight may be due to variation in the thoroughness with which fat and adventitious tissue was trimmed from the pancreas. It is very likely, however, that there was a real difference in the average weight of the organs in the two series of approximately 25 grams. This may be due to factors 1 and 2 mentioned above. Weight differences alone can effect only the yield per pancreas, and not the yield per gram.

5. Difference in method of extraction may very well account for the difference in yield. In our process, for example, considerable insulin activity may have been lost by allowing the pancreatic hash to remain for long periods of time in the extracting fluid.

A rough estimate of the relative efficiency of the two methods was attempted. A single pancreas was ground, and the grindings were divided into approximately equal portions which were then weighed. One portion was extracted by Scott's method. The other portion was extracted by the method described in this paper. All manipulations were carried out as in the preparation of the lot of insulin described earlier, including the elapse of one month, during which the hash stood in the refrigerator after addition of extracting fluid. Seitz filtration was also carried out. The numerous manipulations required by the method we used and the small size of the sample in this instance would probably decrease the yield, if it affected it at all, because the insulin lost on filters and glassware would be proportionately large. Assays were carried out in mice, using 80 to 120 mice for each prepara-

dark green in color, was discarded, leaving a clear, amber-colored supernatant liquid, which was decanted and placed in sterile vials.

Assay

The insulin was first assayed by its ability to produce convulsions in white mice (5). Small numbers (5 to 10 mice) were used for purposes of orientation, and then the human insulin was compared with that of commercial insulin (Lilly US0) in larger groups (46 mice for each preparation). The assay of the final preparation was 21–25 u per ml. by this method. A second assay was done in rabbits using the “cross over” method of Marks (6), which is based on the per cent reduction in blood sugar following the subcutaneous injection of insulin. Blood sugars were determined by the micro-method described by Nelson (7), using the Folin method of protein precipitation (8). Five rabbits were used, each being tested with human insulin and commercial insulin (0.75 units/Kg.). By this method the preparation contained 24 u/ml., the estimated error being approximately 10 per cent for the number of animals used.

RESULTS

A total yield of 2500 units (106 ml. of a solution containing 24 units per ml.) was obtained from 50 pancreases. This represented an average yield of 50 units per pancreas or 0.62 units per gram. The final product was a clear, amber-colored liquid with a slightly greenish hue. It contained 0.34 mg. of nitrogen per ml., only 47 per cent of which could be ascribed to the insulin present. When 6.4 units (0.1 u/Kg.) were given intravenously to a 140-pound human subject with apparently normal carbohydrate metabolism, a normal insulin tolerance curve was obtained. The fasting blood sugar was 71 mg./100 ml.; 15 minutes after the injection it was 39 mg./100 ml.; at 30 minutes, 29 mg./100 ml.; at 45 minutes, 42 mg./100 ml.; at 1 hour, 60 mg./100 ml.; at 1½ hours, 70 mg./100 ml.; and at 2 hours, 105 mg./100 ml.

DISCUSSION

The purpose of this study was to obtain a quantity of human insulin for an experimental study of insulin resistance. Assays on two individual pancreases were made in order to determine what effect the interval between death and processing of the pancreas might have on the yield of insulin. According to rough assays in mice, a pancreas obtained twenty-seven hours after death contained one-quarter as much insulin as one obtained three hours after death.

In studies on the insulin content of the human pancreas from diabetic and nondiabetic individuals, Scott and Fisher (9) found that the nondiabetic pancreas contained an average of 173 units or 1.7 units per gram,

SYMPATHICOTROPIC (LEYDIG) CELL TUMOR OF THE OVARY WITH VIRILISM

REPORT OF A CASE

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MASCULINIZATION due to ovarian tumor is rare, and it is only within the past few years that clinical and laboratory data concerning such cases have been gathered with sufficient completeness to establish some of the diagnostic criteria. Some examples of the condition must still go unrecognized, or pass as states of "functional" amenorrhea or mild hirsutism. In this paper we report a case, cured by surgical removal of the tumor, which presents features of diagnostic interest, clinically and pathologically.

CASE HISTORY

Mrs. D. M., age 46, was admitted to the Montreal Neurological Institute on September 25, 1946. She complained of amenorrhea of eleven years' duration; increased hair growth on the body for six years; loss of libido, nervousness, increased fatigability and a tendency to baldness of the scalp for five years; and huskiness of the voice for one and a half years.

She had been in perfect health and had normal menstrual periods until eleven years before admission. At that time her periods suddenly stopped and she had no bleeding or discharge thereafter. Then an increased growth of hair appeared on her face. Three or four years later hair began to appear on her forearms and legs, and on the chest and abdomen. During the two years prior to admission hair appeared on her back and upper arms and on the back of her fingers, and everywhere it became thicker and coarser. Meanwhile the scalp hair became thin and bald spots appeared. For two years she had noticed that her muscles stood out like those of a man, and that she was much stronger, even though she found she tired quickly. The veins on her arms and legs became larger and more prominent and the skin became leathery. There was a gradual loss of 20 pounds in weight.

About a year and a half before admission her voice began to change, acquiring a masculine, husky quality, and the neck seemed to thicken. Her appetite for food increased so that she might get ravenously hungry, but she was more quickly satisfied than formerly. For a few months there had been mild numbness of the hands and legs. For ten years the patient took regular and nerve-racking electrolysis treatment for removal of facial hair.

In the past five years there had been complete lack of sexual appetite. There was increasing nervousness and selfconsciousness, and a constant indefinable sense of tension

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tion. Scott and Fisher's method yielded 1.6 u/gram of pancreas; our method 1.1 u/gram, or 65 per cent as much. This would indicate that the low yield obtained by us from 50 pancreases, a yield which was only 35 per cent of that obtained by Scott and Fisher, cannot be attributed entirely to the difference in the method used for extraction.

It is possible that a good part of the difference in the yield of insulin per pancreas between our group and that reported by Scott and Fisher (9) was due to the debility of the subjects from whom our pancreases were obtained. If this is true, then the striking difference noted by these observers between the insulin content of pancreases from diabetic and nondiabetic individuals was due only in part to diabetes per se, because in their series the nondiabetic subjects had died suddenly, whereas those with diabetes had died of associated debilitating disease.

SUMMARY

Human insulin suitable for injection into patients was prepared by a method requiring no special apparatus. The yield of insulin was approximately 50 units per pancreas or 0.62 u/gram.

Evidence is presented indicating that the insulin content of the pancreas is reduced in nondiabetic persons dying of debilitating disease.

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The report of the vaginal smear, from the Gyne-cytology Laboratory (Dr. J. E. Ayre) was as follows: "The cytological picture is most unusual. Both vaginal and cervical smears show a complete absence of cornification (endogenous estrogen). The cells are all of an atypical, basal, atrophic type which we have seldom seen. Some of these show multinucleation with foamy appearance throughout the cytoplasm.

"Interpretation: 1. Total absence of cytological evidence of endogenous estrogen. 2. We cannot account for cells of this type. The picture is not typical of that found when male hormone is administered. Whether some adrenal hormones enter this picture we do not know."

TABLE 3. BLOOD ELECTROLYTES

	Plasma		Serum		
	Chloride	N.P.N.	Sodium	Potassium	Calcium
1946	mEq./L	mg. %	mEq./L	mEq./L	mg. %
Oct. 23	—	28.4	—	—	10.5
Oct. 24	101	20.0	142	—	—
Oct. 24	Operation				
Oct. 25	—	—	146	6.3	—
Oct. 28	97	27.5	142	5.0	—
1947					
Oct. 6	—	—	142	4.2	11.2
Oct. 7	101	23.0	142	4.35	11.0

TABLE 4. URINARY OUTPUT OF 17-KETOSTEROIDS, GLYCOGENIC CORTICOIDS AND CREATININE

	17-Ketosteroids	Corticoids	Creatinine
	mg./24 hrs.	units/24 hrs.	Gm./24 hrs.
1946			
Sept. 25 & 26	22.4	—	1.6
Oct. 2 & 3	22.8	63	1.7
Oct. 9 & 10	16.0	74	1.5
Oct. 24	Operation		
Oct. 31 & Nov. 1	10.6	88	—
Nov. 3 & 4	12.9	—	—
1947			
Oct. 7 & 9	10.5	—	1.1

Blood examinations: A glucose tolerance curve (capillary blood) was normal (Table 1). A complete hemogram was normal save for moderate erythrocytosis along with slight leucocytosis, due to an increase in all forms (Table 2). Examination of the electrolytes of the blood showed no abnormality (Table 3).

Hormone excretion: The twenty-four hour output of 17-ketosteroids in the urine was moderately increased, as shown in Table 4. Urinary glycogenic corticoids were within normal limits.

and jitteriness. She was very ill at ease and anxious when in the hospital ward with other women, and asked to be placed in a room by herself.

The family history was noncontributory and there was no history of other endocrine disorder. The patient had had one pregnancy resulting in a boy now 18 years old and alive and well. No subsequent pregnancies had occurred although children were desired.

Physical examination: The patient was a nervous, apprehensive woman of medium height and build. The body contour was masculine, the muscles of the upper and lower limbs stood out strongly like those of a man, and were very powerful. The voice was low but not entirely masculine. Hair over the scalp was thinned and there were several bald patches. Hair over the rest of the body was dark, thick and wiry. It grew especially

TABLE 1. GLUCOSE TOLERANCE CURVE—CAPILLARY BLOOD

	1946	1947
	mg. %	mg. %
Fasting	93	67
1 hour	190	124
2 hours	159	122
3 hours	102	—
4 hours	88	89
5 hours	106	96

TABLE 2. HEMATOLOGY

	1946	1947
RBC	5,500,000	4,300,000
WBC	10,000	8,500
Hb	110%	84%
Color index	1.0	0.98

on the chest, limbs and face. The breasts had lost their normal fullness and the hips were relatively narrow.

There was no evidence of kyphosis or scoliosis of the spine, and there were no striae cerulae. There was no marked evidence of acne. The skin was of normal color and there was no lividity or suffusion. The optic fundi and visual acuity were normal, and the visual fields were full. The remainder of the examination, save for a generalized hyperreflexia, showed nothing abnormal. The blood pressure, measured on many occasions, averaged about 130/84, and the blood Wassermann reaction was negative.

Special examinations: Stereoscopic anteroposterior and right lateral films of the skull showed no evidence of an intrasellar tumor, nor was there any hyperostosis cranialis interna. Stereoscopic films of the lumbar spine showed minimal osteoarthritic spurring, but there was no evidence of intervertebral disc disease nor of osteoporosis. X-ray examination showed a normal chest. Cystoscopy, carried out by Dr. Magnus Seng, and contrast pyelograms failed to show any evidence of adrenal tumor.

Gynecologic examination by Dr. G. Simpson revealed moderate hypertrophy of the clitoris. The remaining organs were small but did not seem atrophic. No evidence of any large ovarian tumor could be made out.

tion, sat in a chair on the second day and was walking on the fourth postoperative day.

Follow-up: No clinical change was noted in the two weeks of observation in the hospital after operation. There was, however, an almost immediate, though modest, drop in the excretion of 17-ketosteroids in the urine, as indicated in Table 4.

The patient was re-admitted in October 1947, one year after operation. She then stated that she had first noted a mental change beginning three weeks after operation. She became progressively more relaxed and lost the indefinable feeling of tenseness. It is of interest to note that at this time no change in her physical appearance was yet



FIG. 2. Cut surface of the ovary and tumor. Bulging, soft dark tumor tissue occupies the center, surrounded by narrow, pale capsule of ovarian tissue.

manifest, to make her feel less selfconscious. In two months the face began to clear of hair and the voice lost its huskiness. Normal menstrual periods were established in three months, after an absence of almost twelve years, and continued regularly thereafter. The skin became softer and smoother and hair decreased over the body and became finer. She gained 20 pounds in weight, mainly on the hips, thighs and breasts. The hair on her head became more glossy. The striking change in appearance is shown in Figure 1. Libido remained low but sexual satisfaction was normal. Pelvic examination showed some remaining hypertrophy of the clitoris and moderate enlargement of the uterus.

Gyne-cytologic examination of vaginal smears now showed normal evidence of endogenous estrogen with glandular and basal cells and some red blood cells and leuco-

A provisional diagnosis was virilism due either to adrenal cortical hyperfunction, or to ovarian tumor. Since both adrenals and both ovaries had to be explored, the Surgeon-in-Chief, Dr. Gavin Miller, elected to use a single abdominal incision. This was done in order to avoid the three incisions which would have been necessary for exploration of the adrenals and pelvic organs separately.

Operation: October 24, 1946. Surgeon: Dr. Gavin Miller.

The abdomen was opened under spinal anesthesia by a midline incision from the xiphoid almost to the symphysis.

The right adrenal gland presented no visible or palpable abnormality. The left adrenal appeared to be considerably enlarged. The right ovary measured approximately

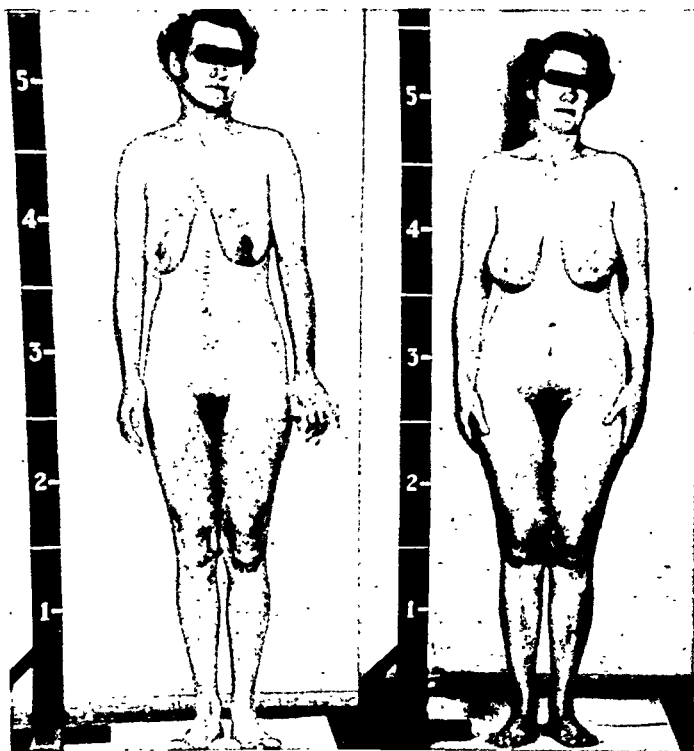


FIG. 1. Appearance of patient immediately after operation (left) and one year later (right). Note change in body contour and loss of excessive hair.

1.5 cm. in diameter and was fibrotic and calcified. The left ovary measured approximately 3.5 cm.; one pole was of meaty consistency, and appeared hyperemic. No other intra-abdominal abnormality was detected. It was not entirely clear whether a lesion was present in the left adrenal or left ovary. The left adrenal was dissected free and removed *in toto*. The left ovary and the fimbriated end of the fallopian tube were also removed. The abdomen was closed in layers without drainage, using steel wire.

The patient's postoperative condition was satisfactory. Five per cent glucose and plasma were administered during operation. Following operation, adrenal cortical extract was given, 10 cc. intramuscularly every four hours for three days, and the patient was kept on a low potassium diet. She sat up on the side of the bed one day after opera-

tumor tissue from the ovarian tissue. At the hilus of the ovary, the capsule of ovarian tissue was lacking and the tumor cells were bordered by the connective tissue of the mesovarium. The tumor was composed of polyhedral cells, arranged in strings, columns or in apparent haphazard manner. At no point in the tumor could any suggestion of tubular adenomatous structure be observed. A loose network of fibrous tissue accompanied the vessels which penetrated the tumor obliquely from the periphery. Thin spokes of fibrous tissue radiated from the vessels, disappearing between the cells of the neoplasm. The tumor cells (Fig. 4) had round or finely indented nuclei of lymphocyte size and occupied

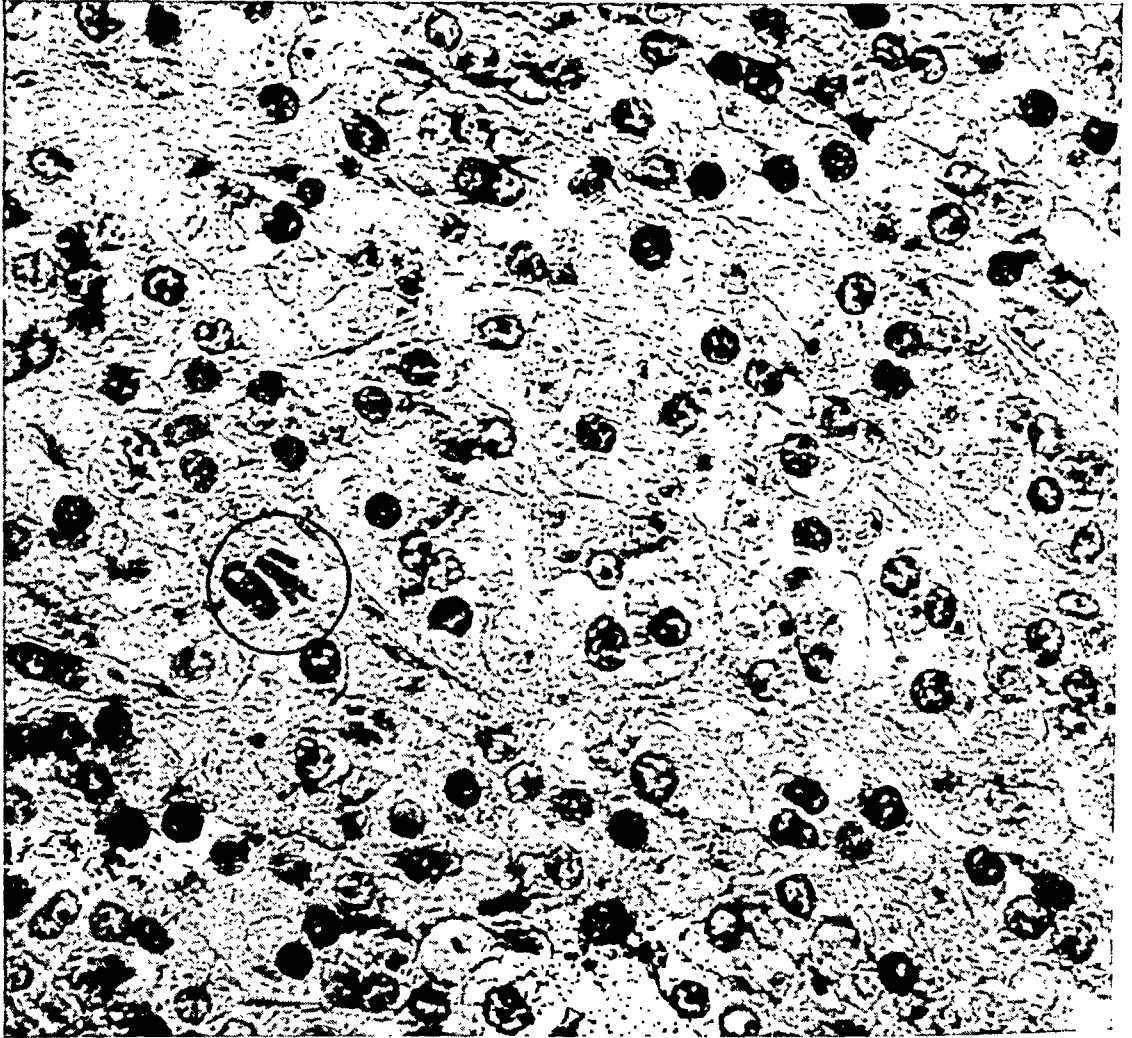


FIG. 4. A cell containing three characteristic crystalloids has been circled. Masson's trichrome stain. $\times 640$.

about half the cell diameter. Nuclear membranes were distinct and the chromatin tended to be concentrated at the nuclear periphery. Most nuclei had a distinct, rather large, basophilic nucleolus. The cytoplasm was acidophilic, finely granular, and cell borders were usually sharp. In many of the cells there was a condensation of homogeneous cytoplasm about the nucleus, while at the cell periphery the structure was looser and paler. Mitoses were rare. No pigment was noted in the cytoplasm and vacuolation was not a feature. Many of the cells, especially near the periphery of the tumor, contained a bar-

cytes. The blood sugar curve was normal (Table 1). The moderate erythrocytosis and leucocytosis had disappeared (Table 3). The output of 17-ketosteroids in the urine remained normal (Table 4).

PATHOLOGIC EXAMINATION

The removed ovary measured $3.7 \times 3 \times 3$ cm. and weighed 10 Gm. (Fig. 2). Its external surface was smooth, glistening and pale bluish grey in color. It had a soft rubbery consistency. Sections through its longest axis showed yellow-white ovarian tissue forming a capsule 2-4 mm. in thickness about a central tumor mass. The capsule of ovarian

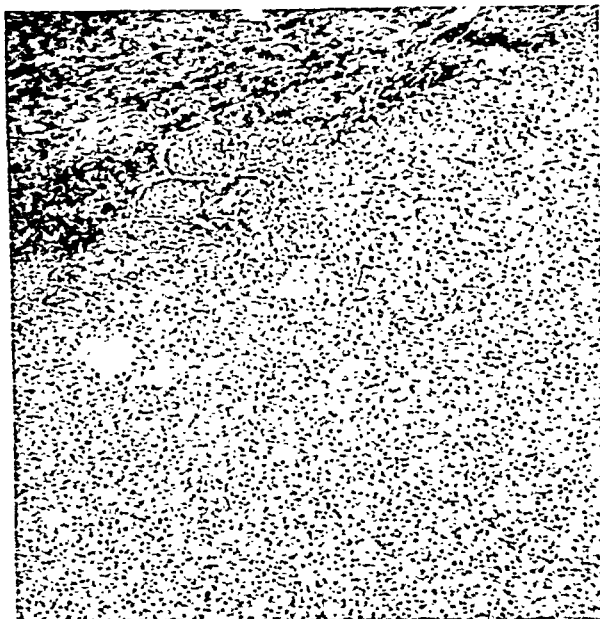


FIG. 3. Microscopic appearance of the tumor. Ovarian stroma containing thick-walled blood vessels occupies the upper left half of the picture and the neoplasm the lower right. Note absence of any fibrous capsule. Hematoxylin and eosin stain. $\times 119$.

tissue appeared incomplete at the ovarian hilus. No cysts were grossly visible in the ovarian tissue. The cut surface of the tumor bulged irregularly and consisted of soft, rather friable red-brown tissue having a coarsely lobular architecture. Irregular hemorrhagic zones, up to 0.7 cm. in diameter, were present in the central portion. At the periphery some of the tumor tissue showed indistinct yellow areas.

The left adrenal was unaltered in shape. When stripped of fatty tissue, it weighed 8.5 Gm. and measured $7 \times 3 \times 1.5$ cm. The cortex was deep yellow and the medulla red-brown, and neither was considered grossly abnormal.

Microscopic examination: Blocks were taken from various regions of both specimens. These were fixed in neutral formalin, and sections were stained with hematoxylin and eosin and Masson's trichrome; stains for reticulum and fat were also made.

The capsule of the ovarian tumor was found to consist of ovarian stroma in which corpora albicantia but no follicles were seen. No fibrous capsule (Fig. 3) separated the

part of the mesovarium. They occur singly or in small collections, between the nerve fibers, or closely applied to the perineurium. They are usually in the immediate vicinity of a small blood vessel. There is considerable variation in size and shape from cell to cell. The cells usually are polyhedral in outline, and show a tendency to be arranged in short columns or small alveoli. They have a granular cytoplasm, which may show condensation about the nucleus, whereas the periphery is more pale staining and loose. Cell borders are fairly distinct. Within the cytoplasm small globules of fat may be found, and there is often a finely granular brown pigment. The most distinctive, though inconstant cytoplasmic inclusions are relatively large bar-shaped crystalloids with oblique ends. These occur singly or in groups of two or three within a single cell. The crystalloid usually fills the length of the cell, and may be of sufficient width to push the nucleus to one side. As a rule, it is surrounded by a clear zone of cytoplasm. Morphologically and microchemically these crystalloids are indistinguishable from the albuminoid crystalloids of Reinke, found in the interstitial cells of the testis.

These cell collections had been previously observed by von Winiwarter in 1910 (3) who held the opinion that they were pheochrome elements. Berger has been the chief proponent of the view that these cells are morphologically identical with the interstitial (Leydig) cells of the testis and has suggested that they may be the source of male hormone, which can be extracted from the ovary (4).

The cells reach their fullest development in the adult premenopausal ovary, but small groups of cells which are probably of identical nature, have been found in the ovaries of infants (5), and their senile remnants have been demonstrated in postmenopausal ovaries (2).

We believe that the tumor in this case exhibits morphologic features which identify it as a neoplasm of sympathicotrophic cells (Leydig cells). Sympathicotrophic cells are most numerous in the region of the ovarian hilus. The interruption of ovarian encapsulation of this tumor in the hilus is in keeping with origin from sympathicotrophic cells in this region, with subsequent extension into the ovarian medulla. The cell detail and arrangement, together with the presence of the characteristic crystalloids, are typical of sympathicotrophic cells, and argue against the possibility of origin from theca cells, lutein cells or adrenal rest tissue. The removed adrenal exhibits no abnormal features which warrant its consideration as the cause of virilization.

The remarkable clinical remission following the removal of the ovary indicates an endocrine influence ascribable, we believe, to the sympathicotrophic cell tumor within it. This is in keeping with the suggestion by Berger (4) that the sympathicotrophic cells of the ovary are a) morphologically identical with the interstitial cells of the testis, and b) may be the source of male hormone which can be extracted from the ovary.

shaped body with oblique ends, staining an amber color in Masson's trichrome preparation. This structure extended to the longest diameter of the cell and in some instances appeared to push the nucleus to one side, compressing it into an oval shape. Usually these crystalloids were surrounded by a narrow clear zone. Occasional cells were seen containing two or three of these bodies, lying roughly parallel to one another. In sections stained with haematoxylin and eosin the crystalloids were acidophilic, and appeared to merge with the cytoplasm of the cells containing them, so as to render their identification extremely difficult. Reticulum stains showed a delicate reticulum extending between the cells of the tumor. Frozen sections, stained with Scharlach R., revealed fine lipid droplets in some of the cells; the majority, however, were fat-free.

Sections of the adrenal showed both cortex and medulla relatively broad and well preserved. The architecture and cellular detail of both regions were considered normal.

On the basis of the morphology of the tumor, it was designated as a tumor of the sympathicotrophic cells of the ovarian hilus. There was no evidence that the tumor was malignant.

COMMENT

Clinical findings: Pituitary basophilism had to be excluded in this case. This was done on the basis that certain features were lacking; *viz.*, striae cerulae, osteoporosis, painful adiposity, vascular hypertension and increased glycogenic corticoids. The moderate increase in blood elements was interpreted as a normal male pattern rather than a true polycythemia, and it reverted to a normal female picture after operation. The remaining features, amenorrhea, increased hair growth, clitoral hypertrophy and other signs of masculinization pointed to either adrenal cortical hyperfunction or to a virilizing ovarian neoplasm. We also feel that the emotional aberrations were part of the endocrine picture.

This case also illustrates that a virilizing ovarian tumor is not necessarily associated with a greatly increased excretion of 17-ketosteroids in the urine. The average normal 24-hour output for a woman of this age is 11.0 mg. as determined at our clinic (1). Our patient excreted about 20.4 mg. per day before operation, and a normal amount thereafter. Very high outputs have been described hitherto only in cases of adrenal cortical tumors or hyperfunction. However, a normal output does not rule out either adrenal or ovarian tumor.

In a case such as this, when precise localization of the tumor cannot be arrived at on clinical findings, it is necessary to consider exploration by means of a large abdominal incision, in order that both adrenals and ovaries may be investigated at the one time.

Pathologic findings: In 1923 Berger (2) drew attention to certain cell groups in the hilus of the ovary, which, because of their intimate association with sympathetic nerve fibres of the region, he named sympathicotrophic cells. The cells occur in the hilus of the ovary and in the mesovarium, being particularly frequent in the rete ovarii, and in the median

Addendum

Since this article was submitted for publication, a report of two similar cases has been published by William H. Sternberg (*Am. J. Path.* 25: 493 (May) 1949).

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It seems to us advisable to separate this ovarian tumor from the rather heterogeneous group of "arrhenoblastomas," and to apply to it the designation indicating its cells of origin. In doing so we have adhered to the name originally applied by Berger (2). It would perhaps be justifiable to refer to such tumors as Leydig cell tumors, or interstitial cell tumors of the ovary, names, which may be more indicative of the true nature of the cells. However, until a sufficient number of cases have been recorded for accurate analysis, it seems to be advisable to retain the designation of sympathicotropic cell tumor, which appears to have chronologic priority over other terms.

It is interesting to note that Selye (6), in discussing "arrhenoblastomas" states: "It is quite possible that the degree of Leydig cell development within the tubular adenomas could account for the virilization seen in some cases, but this has not yet been proven." The present case, in which no tubular structure was observable, appears to be in line with such a supposition.

Sympathicotropic cell tumors with virilization have been reported previously by Berger (4) and Dreyfus and Barrozo do Amaral (7). Berger's case was a woman of 50 years, who had symptoms of facial hypertrichosis, male pubic escutcheon, deep, hoarse voice, atrophy of the breasts, and marked enlargement of the clitoris, but who had a normal menstrual cycle. The symptoms had been present for eighteen years, but showed significant regression after removal of her uterus and ovaries for uterine fibromata.

Berger expresses the opinion (8) that the tumors described by Cosacresco *et al.* (9) and by Giordano and Haymond (10), whose sections he has examined, are derived from the same cells.

In discussions of masculinizing ovarian tumors, neoplasms of sympathicotropic cells are rarely mentioned.

Since so few cases of this rare neoplasm have been reported, it is felt that the addition of the present case may help to establish it as an entity.

SUMMARY

Clinical and pathologic features of a case in which there was a tumor of the ovarian sympathicotropic cells are described. Attention is drawn to the fact that this rare neoplasm is frequently overlooked in consideration of virilizing ovarian tumors.

Acknowledgment

The authors are indebted to the late Dr. Louis Berger, who kindly examined our material and suggested the diagnosis. Thanks are also due to Professor Theodore R. Waugh and Professor G. Lyman Duff for assistance in preparation of the material.

TABLE 1. OBSERVATIONS WHICH SUGGEST A POSSIBLE RELATIONSHIP OF SEX
HORMONES TO GOUT AND TO URATE METABOLISM

(The observations on which these statements are based are
reviewed in detail elsewhere (3, 4, 5, 6).)

-
1. Most reported series of gout patients consist from 75 to 95 per cent of men.
 2. Eunuchs are reputed to be free from gout.
 3. In women, gout generally does not have its onset until shortly before or at the menopause.
 4. In premenopausal gouty women, attacks of gouty arthritis frequently are reported to begin at the menses.
 5. The average plasma urate level of normal adult men is higher than that of normal adult women.
 6. The average plasma urate of gouty men is higher than that of gouty women.
 7. In normal children, the average plasma urate level is lower even than that of normal adult women. The sex differential in plasma urate appears at puberty and is not present in normal children.
 8. Men who inherit asymptomatic hyperuricemia do not generally show elevated urate levels until after puberty.
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Methods for determining 17-ketosteroids which do not include the separation of the neutral ketonic fraction with Girard T reagent may give results higher than those reported here. The Girard T separation eliminates nonketonic chromogens which are apparently disproportionately high in the urine of gouty patients.

RESULTS

17-ketosteroid excretion in gout: Table 2 shows the greatly diminished output of 17-ketosteroids observed in all 11 gout patients. The average of 3.24 mg./per day for the 10 men in this table is about one-third of the minimum normal value. We know of no other reported study of ketosteroid excretion in gout,¹ but Dr. William Wolf has told one of us (W.Q.W.) of observing moderately reduced 17-ketosteroid excretion in this disease.

The 5 male patients whose age was over 45 years had a mean daily 17-ketosteroid excretion of 2.79 mg. The 5 younger gouty males had a mean output of 3.64 mg. The known decline in ketosteroids with age (9) presumably accounts for this small difference.

¹ Urinary androgens were estimated biologically, but 17-ketosteroids were not determined in the patient with male pseudohermaphroditism(?) and typical gout reported by Rosenberg in 1942(7). Such patients are ordinarily found to have 17-ketosteroid outputs and urinary androgen excretory levels considerably above normal. The urinary androgen activity in this patient was equivalent to 2-8 mg. of androsterone per day. This is within normal limits for normal adult males, but distinctly low for patients of this type (8).

AN ENDOCRINE FINDING APPARENTLY · CHARACTERISTIC OF GOUT:

VERY LOW URINARY 17-KETOSTEROID EXCRETION WITH CLINICALLY NORMAL ANDROGENIC FUNCTION*

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ROSENBERG, M.D.

with the technical assistance of

K. KADOTA, B.S. AND L. TURNER, B.S.

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WE HAD speculated on the role of androgens (Table 1) and other hormones in gout but deferred study of the problem because most gout patients are free of obvious endocrine disease. One of our gout patients, a vigorous alert 59-year-old tavern keeper, who complained of impotence and hair loss on the forearms, was then found to have a urinary 17-ketosteroid excretion of only 0.7 mg./day. In current endocrine theory, this finding implies either failure of both gonadal and adrenal androgen production, or a more obscure disturbance in steroid production or metabolism. This observation led to the survey of a group of gout patients reported here. Unusually low values for 17-ketosteroid excretion were found in all patients studied. An attempt will be made to explain this finding by detailed consideration of the various known causes for very low 17-ketosteroid outputs.

Material and Methods

The data were chiefly obtained from study of the 11 patients listed in Table 2. Data from other patients seen during the past few years have been quoted in the discussion. Certain other investigators, whose aid is acknowledged elsewhere in the text, have provided important related data.

The urate values are for total urates, determined colorimetrically. Normal values do not exceed 6.2 mg. per cent (1). 17-Ketosteroids were estimated by the procedure of Talbot, Butler and MacLachlan (2).

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** These Departments are in part supported by the Michael Reese Research Foundation.

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Abnormally decreased ketosteroid excretion occurred both during acute gouty arthritis and in asymptomatic interval gout. It would appear that neither the activity of the disease nor the severity of the constitutional reaction are responsible for the low ketosteroid output.

17-ketosteroid excretion in non-gouty hyperuricemia: We attempted to determine if the diminished 17-ketosteroid output was a direct consequence of hyperuricemia. Table 2 shows that 3 of 4 relatively healthy subjects

TABLE 2. BASIC DATA ON GOUT PATIENTS STUDIED FOR HORMONAL STATUS AND ON PATIENTS WITH NON-GOUTY HYPERURICEMIA

Patient	Sex	Age, years	Height, inches	Weight, pounds	Average serum urate, mg. %	B.M.R. %	17-ketosteroids, mg./24 hrs.*	Glomerular filtration rate, cc. per minute per 1.73 sq. M.	Hepatic function tests**
<i>Chronic gouty arthritis; remission</i>									
M.J.	F	48	59	133	13.3	+12	0.67	54	Normal
O.T.†	M	59	66	168	12.1	- 5	0.96	72	Normal
<i>Interval gout</i>									
S.K.	M	49	71	165	7.7	+ 3	0.69	90	Normal
W.S.	M	51	66	160	9.4	—	3.60	75	Normal
H.N.	M	49	54	144	7.6	—	5.90	86	Normal
M.P.	M	43	69	132	7.7	+ 5	6.60	74	Slight impairment
E.W.	M	23	70	178	6.6	—	1.74	116	Normal
A.S.	M	43	72	190	8.4	—	2.25	—	Normal
<i>Acute gouty arthritis</i>									
M.K.	M	37	70	270	10.5	-9†	2.24	—	Normal‡
R.H.	M	44	68	200	7.2	—	5.35	—	Normal‡
B.V.	M	54	68	180	10.2	—	3.02	—	Normal‡
<i>Average, all gout patients</i>									
		45	68	175	9.2	1	3.00	81	
<i>Non-gouty hyperuricemia</i>									
TOB.‡	M	36	71	246	7.5	+ 8	4.3	—	Normal
KWI.‡	M	39	68	290	7.1	—	12.0	—	Normal
WOL.‡	M	30	73	215	6.4	- 5	10.0	121	Normal
SCH.§	M	39	67	145	6.3	—	18.5	107	Normal

* Normal values for urinary 24-hour 17-ketosteroid excretion:

Females: 7.0 to 12.0 mg. per 24 hours.

Males: 9.0 to 25.0 mg. per 24 hours.

** The group of tests used included: serum bilirubin, serum total protein and protein fractionation, serum cholesterol and cholesterol partition, thymo, turbidity, thymol flocculation and cephalin-cholesterol flocculation. In some cases, colloidal gold flocculation, alkaline phosphatase and bromsulfalein clearance were also determined. *Normal* indicates no abnormal result on any test.

† Complaints of impotence and hair loss on forearms; azoospermia found. Good response of both complaints to administration of testosterone propionate, 25 mg. parenterally, three times weekly, in about 6 weeks.

‡ These determinations were carried out during the interval stage.

§ Diagnosis: possible hypothalamic(?) hyperuricemia. This diagnosis is applied to a group of 7 patients in whom unexplained hyperuricemia has accompanied signs and symptoms suggestive of hypothalamic disorder. The symptomatology has included: obesity (7 patients), R.B.C. over 5.0 million (5 patients), hypersomnia (5 patients). Additional findings in individual patients have been: hypercholesterolemia (326 mg. %), electrolyte changes resembling those of Cushing's syndrome, intermittent hypochloremia, familial pitressin-resistant diabetes insipidus with normal renal function tests, unexplained eosinophilia and lymphocytosis, habitual oliguria, and syndactyly. A case resembling KWI has recently been published by Mussio Fournier and Proto (10).

§ Cystinuria, familial.

with non-gouty hyperuricemia excreted normal amounts of ketosteroid. The size of the group and the degree of elevation of plasma urate are not optimal because of the relative infrequency of suitable subjects. The results do suggest that 17-ketosteroid excretion is not necessarily diminished in non-gouty hyperuricemia.

TABLE 3. IN MALES WITH RHEUMATOID ARTHRITIS OR SPONDYLITIS,
AVERAGE DAILY 17-KETOSTEROID EXCRETION IS
NOT SO LOW AS IN GOUT

(Data on patients with rheumatoid arthritis and with spondylitis are unpublished results made available by Dr. A. P. Forbes and Dr. W. S. Clark of the Massachusetts General Hospital, Boston. For the purposes of classification, the one male and the one female patient with chronic gouty arthritis in remission have been considered to be in the interval phase.)

	Males		Females	
	Num- ber	17-ketosteroids mg./24 hrs.	Num- ber	17-ketosteroids mg./24 hrs.
<i>Normal Adult, average values</i>				
Massachusetts General Hospital	—	12.5	—	8.2
Michael Reese Hospital	—	12.0	—	9.0
<i>Rheumatoid Arthritis (Massachusetts General Hospital)</i>				
Rheumatoid polyarthritis	4*	9.7	11	4.6
Rheumatoid spondylitis	3	10.5	1	5.2
All cases	7	10.0	12	4.7
<i>Gout (Michael Reese Hospital)</i>				
Interval gout	7	3.1	1	0.7
Acute gouty arthritis	3	3.5	—	—
All cases	10	3.2	1	0.7

* The 17-ketosteroid outputs in two men with minimal constitutional signs were 11.0 and 11.0 mg. per 24 hrs. respectively. The two remaining patients, with more severe constitutional signs, had average 17-ketosteroid outputs of 10.5 and 6.4 mg. per 24 hours.

17-ketosteroid excretion in rheumatoid arthritis: It appeared important to compare the ketosteroid excretion of gouty men with that of men with rheumatoid arthritis and spondylitis to see whether diminished 17-ketosteroid excretion was specific for gout. This was made possible when Dr. Anne P. Forbes and Dr. William S. Clark of the Massachusetts General Hospital very generously made their unpublished observations available.

Table 3 summarizes comparative findings in gout and rheumatoid ar-

Abnormally decreased ketosteroid excretion occurred both during acute gouty arthritis and in asymptomatic interval gout. It would appear that neither the activity of the disease nor the severity of the constitutional reaction are responsible for the low ketosteroid output.

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§ Cystinuria, familial.

In gout, 17-ketosteroid excretion is low even during the interval stage when the disease is apparently completely quiescent, except for hyperuricemia. Comparable male patients with rheumatoid arthritis of low activity have normal ketosteroid outputs. The presence of diminished 17-ketosteroid outputs in all stages of activity would appear to be peculiar to gout.

Other causes of very low 17-ketosteroid outputs: Table 4 summarizes relevant conditions which regularly produce 17-ketosteroid outputs below about 2.5 mg./day, and groups these causes according to the responsible underlying disturbance in function. In females virtually all androgen originates in the adrenal cortex and decreased ketosteroid excretion implies decreased adrenal androgen production or a metabolic abnormality. In males, androgen is produced by both testis and adrenal. Ketosteroid outputs below 2.5 mg./day, in the absence of an anomaly of metabolism, result only from decreased androgen production by both these glands (14-17).

Five men and the one woman in our series had 17-ketosteroid outputs below 2.5 mg. per day. In at least these 6 patients, one may assume the cause to be either deficiency in both adrenal and gonadal androgen production, a qualitative abnormality in the pattern of steroids produced, or a disturbed intermediate steroid metabolism. Each of these possibilities will be considered.

Adrenal, pituitary and thyroid insufficiency: The average daily excretion of 17-ketosteroids is regularly below 2.5 mg./day in panhypopituitarism, in females with Addison's disease, in those males with Addison's disease in whom hypogonadism develops, and in severe hypothyroidism or myxedema (15, 16, 18- through 31).

Table 5 compares certain clinical and laboratory findings in adrenal, thyroid or pituitary insufficiency with those in interval gout. This table indicates that the diminished 17-ketosteroid excretion in gout cannot be referred to thyroid, pituitary, or adrenal failure.

Liver function: 17-ketosteroid excretion is decreased in severe chronic liver disease, probably as a result of a number of endocrine mechanisms (30, 32).

Table 2 summarizes the results of testing the 11 gout patients with a battery of sensitive liver function tests. With one minor exception, the results were entirely normal, a finding consistent with that obtained when we applied the same tests to a larger group of gout patients (33). In general, the results indicated that abnormalities in hepatic function were unusual in gout unless complicating disease were present.

Normal results with function tests did not eliminate the possibility that a specific enzymatic deficiency of the liver might have resulted in disturbed steroid metabolism and in low ketosteroid outputs. Fortunately a direct test of this possibility was available. Following intramuscular injection of

TABLE 4. CONDITIONS ASSOCIATED WITH VERY LOW 17-KETOSTEROID EXCRETION

DECREASED PRODUCTION OF 17-KETOSTEROID PRECURSORS	
<i>Associated with decreased 11-oxysteroid production</i>	
1. Pituitary failure	
2. Severe hypothyroidism and myxedema	
3. Adrenal failure in females	
4. Adrenal plus secondary gonad failure in males*	
5. Severe hepatic dysfunction	
<i>Associated with increased 11-oxysteroid production</i>	
1. Cushing's syndrome; some cases**	
2. Alarm reaction, "resistance stage"	
ABNORMAL TYPE OF STEROID PRODUCTION OR METABOLISM	
<i>Normal precursor not converted to 17-ketosteroid</i>	
1. Severe hepatic dysfunction	
<i>?Androgen produced not 17-ketosteroid precursor?</i>	
1. Arrhenoblastoma, some cases	
2. Some cases of hirsutism in females†	

* Pure testicular failure is not included in this table, since it is not believed to result in 17-ketosteroid outputs below 50% of minimum normal values.

** Cushing's syndrome actually seldom leads to very low values. However Albright (13) has argued for a primary increase in oxysteroid and decrease in ketosteroid production, making Cushing's syndrome analogous to the "resistance stage." He explains the normal to moderate elevation in ketosteroid excretion of many patients either as compensatory or, in the case of tumors, as mixed types of syndromes.

† The status of pure hirsutism without virilization as an androgenic manifestation is not firmly established. Hypersensitivity of the end-organ might be responsible. Moreover, some types of hirsutism occur in Cushing's syndrome when no other suggestion of virilization is present.

thritis. The tendency of gouty men to have lower 17-ketosteroid outputs than men with rheumatoid spondylitis or polyarthritis is apparent. The values in rheumatoid arthritis are consistent with those reported by Davison, Koets and Kuzell (11), whose data even suggest that ketosteroid excretion is occasionally high in rheumatoid arthritis.²

² Pedersen-Bjergaard and Tønnesen (12) have recently reported results of urinary androgen assays by the capon comb method in various groups of male patients, including 17 with chronic rheumatoid arthritis. Normal values were found to be from 5 to 25 c.u./24 hrs. Men with prostatic hypertrophy and impotence had outputs within the normal range. The average androgen excretion in patients with rheumatoid arthritis was low, being approximately 5 c.u./day. It did not exceed 8 c.u./day in any of the 17 analyses.

50 mg. of testosterone propionate, normal males excrete 14 to 70 per cent as extra urinary ketosteroid within seventy-two hours. Inability to convert injected testosterone to ketosteroid is one of the abnormalities responsible for low ketosteroid excretion in cirrhotics. In 4 patients with Laennec's cirrhosis, Lloyd and Williams (32) recovered only 0 to 7 per cent of the injected testosterone as ketosteroid in the seventy-two hours after administration.

TABLE 6. 17-KETOSTEROID EXCRETION FOLLOWING INJECTION OF 50 MG. OF TESTOSTERONE PROPIONATE SHOWED NO ABNORMALITY IN THE CONVERSION OF ANDROGEN TO 17-KETOSTEROID

(Normally, 14 to 70 per cent of the injected material is recovered as urinary 17-ketosteroid within 72 hours of the time of injection. The average increase in urinary 17-ketosteroids per day during this postinjection period is normally between about 3 and 12 mg./day when estimated on the pooled 72-hour specimen. Urine samples in the two experiments below were only 48-hour collections, but despite this, the recoveries were normal.)

	17-Ketosteroid excretion		Recovery
	Before testosterone mg. per day	After testosterone mg. per day	Per cent in 48 hrs.
Patient O.T.	1.0	10.0	36%
Patient S.K.	0.7	10.3	38%

Table 6 summarizes results obtained when 50 mg. of testosterone propionate was administered intramuscularly to 2 gouty men whose basal 17-ketosteroid excretions were 1.0 mg. per day or less. The normal recoveries of 36 per cent and 38 per cent in forty-eight hours indicate unimpaired ability to convert testosterone to ketosteroids.

Renal function: Renal function was sufficiently well preserved to render "retention" of 17-ketosteroids unlikely. Glomerular filtration rate averaged 81 cc./min./1.73 sq.M. (Table 2), which is somewhat higher than the average value of 64 cc./min./1.73 sq.M. obtained in 20 unselected gout patients (unpublished data). This value represents only moderate renal impairment and is accompanied neither by clinical signs and symptoms of renal insufficiency nor by biochemical evidence of renal impairment such as elevations in serum urea or inorganic phosphate. The results of the testosterone injections, noted above, also suggest that when ketosteroids actually were produced, they could be excreted without difficulty.

TABLE 5. SOME OBSERVATIONS WHICH INDICATE THAT THE LOW 17-KETOSTEROID OUTPUTS OF PATIENTS WITH GOUT CANNOT BE EXPLAINED AS THYROID, ADRENAL, OR PITUITARY FAILURE

(The statement concerning life expectancy in gout as compared with the non-gouty obese may be deduced by comparing Bauer and Klemperer's remarks on the prognosis of gout (34) with those of Evans on the prognosis of obesity (35). For a recent, but characteristic, statement on the accomplishments of the gouty, see McCracken, Owen and Pratt (36). The table deals with the findings in interval gout, because no complicating inflammatory lesion is present in these patients.)

Some signs and symptoms of thyroid, adrenal, or pituitary failure	Findings in interval gout patients
1. Anorexia	Obesity usual; average 20 pounds over weight (37)
2. Asthenia	Unusual vigour and accomplishment usually noted by reviewers (36)
3. Hypotension	Hypertension in about 50 per cent (37)
4. Fasting hypoglycemia	Never reported. Diabetes mellitus in about 2 to 6 per cent
5. Tendency to Addisonian crisis	Never reported
6. Decreased life expectancy	Life expectancy equal to that of equally obese non-gouty
7. Addisonian pigmentation	Never reported
8. Tendency to increased serum potassium, diminished sodium and chloride	Not present ((38), p. 96, table 1)
9. Decreased B.M.R.	B.M.R. is normal (Table 1)
10. Hypercholesterolemia (chiefly in hypothyroidism)	Serum cholesterol is usually normal in interval gout (33)
11. Anemia	Average values; R.B.C. 5.08 million, hematocrit 46, hemoglobin 15.7 Gm. per cent (37)
12. Lymphocytosis	Average values: 26.7 per cent of total leukocytes; 1924 per cu. mm. (37)
13. Tendency to eosinophilia	Average values: 2.6 per cent of total leukocytes; 181 per cu. mm. (37)*

* Slide differential

day in the male cannot be explained solely as testicular deficiency but must be interpreted as dual failure of adrenal and gonadal androgen production, or as resulting from an abnormal pattern of steroid secretion or metabolism (14-17). All low outputs in the female must be referred to deficient adrenal androgen production or to metabolic error. Six patients in this series were found to have a 17-ketosteroid excretion below 2.5 mg./day.

Pituitary insufficiency, Addison's disease in females, Addison's disease with secondary testicular failure in males, and severe hypothyroidism regularly produce comparable decreases in 17-ketosteroid output. There are, however, no findings in gout which suggest obvious adrenal, pituitary, or thyroid insufficiency. We also considered the possibility that hepatic impairment, either generalized or as a specific enzyme deficiency, might be responsible. A battery of sensitive liver function tests gave no evidence of diffuse hepatic impairment in these or other gout patients (33). Testosterone propionate, administered parenterally, was recovered as urinary ketosteroid in the usual amounts. Since testosterone is now believed to be the most important physiologic androgen (41), normal recoveries after injection appeared to eliminate an isolated hepatic enzyme defect which might prevent metabolism of testosterone to ketosteroid. Renal function was sufficiently well preserved to render unlikely the theoretical "retention" of 17-ketosteroids.

A remaining possibility is that the endocrine situation in gout corresponds to the "resistance stage" of Selye's general adaptation syndrome (42). The "resistance stage" may be roughly defined as the phase of convalescence from acute injury or the state of dynamic equilibrium during chronic injury or disease, in which the organism manifests increased tolerance to a repetition or to an increase in intensity of the original injurious stimulus. It is partially characterized by increased corticoid (glycocorticoid, 11-oxysteroid) production and excretion, and by a concomitant reduction in 17-ketosteroid production and excretion (20, 24, 26, 27, 28, 29, 31).

Patients who exhibit the characteristic steroid excretion pattern of the "resistance stage" are generally chronically ill or are convalescing from severe illness or operation. The gout patient, on the other hand, may exhibit his characteristic low 17-ketosteroid excretion during the interval phase when he is apparently entirely well, except for hyperuricemia. Good health alone does not prove the absence of "resistance stage" endocrine status in interval gout, since the gouty conceivably might exemplify good adjustment to chronic injury.

We have reported preliminary attempts to evaluate this problem indirectly by study of a number of metabolic variables known to reflect the rate of adrenocortical glycocorticoid production (37). This and other re-

Biologic androgen activity: 17-ketosteroids are not the sole end-product of androgen metabolism; and, in addition, certain 17-ketosteroid precursors, such as etiocholanolone, are not potent androgens. Nevertheless, androgenic function and 17-ketosteroid excretion are moderately well correlated under most circumstances, and a 17-ketosteroid output of less than 2.5 mg. per day in a mature adult man will generally be associated with obvious evidence of deficient androgen activity.

There is little about gout as a disease (Table 1), or about the gouty as individuals, to indicate that their diminished ketosteroid excretion actually is associated with the expected hypogonadism. The only two reports of hypogonadism in gout are those of Lichtwitz (39) and of Hench (40), but neither case can be considered spontaneous hypogonadism. In the former, hypogonadism followed eight years of severe polycythemia vera and, in the latter, mumps orchitis during adolescence was apparently responsible.

Good evidence indicated that the patients had had normal gonadal function throughout most of adult life and some findings suggested that it remained normal when the low 17-ketosteroids were found. Nine of the 10 men were married. Six of the married men had children: 2 had one child each, 3 had two children each, and 1 had four children. Five of the 9 men over 30 exhibited definite advanced bitemporal baldness, while 1 showed a milder degree. Creatine and creatinine excretion were studied in 6 men (Table 7). The creatinine coefficient, an index of functional muscle mass, averaged 21.1 mg./Kg./day, well within the normal limits of 20 to 26. Significant creatinuria of above 1.0 mg./Kg./day was absent in 4 of the 6 patients. These observations are presumptive evidence for normal androgenic status rather than absolute proof. Further documentation in the form of sperm counts, FSH assays, androgen outputs and similar data is now being sought.

DISCUSSION

The data detailed in this article may be summarized briefly. In gout, the average daily outputs of urinary 17-ketosteroids are below half of the minimum normal values. This decrease appears not to depend to any important extent upon the stage of the disease, or upon the age of the patient. It is present in entirely asymptomatic patients with interval gout. Since it does not uniformly occur in non-gouty hyperuricemia, it may not be a necessary consequence of hyperuricemia. Low 17-ketosteroid outputs do not occur in men with mild rheumatoid arthritis. The decreased ketosteroid excretion is therefore a property of gout itself and not a general phenomenon of the arthritides.

Decreased 17-ketosteroid excretion in gout is not satisfactorily explained by any of the currently accepted mechanisms. Outputs below 2.5 mg. per

TABLE 7. SOME BIOLOGIC INDICES OF ANDROGEN ACTIVITY IN GOUT PATIENTS

(Fertility data, particularly in regard to children born after the onset of clinical symptoms, are partly those assembled by Dr. C. W. Cotterman from unpublished data of Smyth, Cotterman, and Freyberg. The table includes male patients only; female patients will be discussed in a later communication.)

	<i>Yes</i>	<i>No</i>
Married?	32	5
If married, any children?	27	5
Any children born after onset of clinical gout?*	6	21
Bitemporal baldness?†	50	45
Significant creatinuria?‡	3	10

Creatinine coefficient

	Mg./Kg./day
Average, normal adult males	20.0-26.0
Average, 13 gout patients	20.7

Fertility of male gout patients

Number of children	Number of patients
0	5
1	10
2	11
3	0
4	4
5	0
6	1
7	1

Average number of children per patient: 1.9

* The average age at the first reported gout attack was 41 years, an unusually late age. It would seem probable that some of the patients may not have recalled their very first attack, hence that the number of patients siring children after the first attack is underestimated in this table.

† Well established baldness in 33; thinning to mild in 17.

‡ Defined as an excretion of creatine in excess of 1.0 mg. per Kg. per day.

The clinical data suggest the low 17-ketosteroid excretions of the gouty to coexist with normal biologic androgen activity. There are a few excep-

tion of sufficient adrenocorticotropin (49) and are believed to be referable to the demonstrable release of preformed adrenal androgen by this hormone. Exacerbations of skin lesions as gouty prodromata were frequently commented upon in the older literature (39), and a form of "gouty eczema" was believed to occur. Talbott (36) found evidence of psoriasis or eczema in less than 10 per cent of his series. However, those patients who did have either of these conditions reported an exacerbation of local skin symptoms before or during an attack of acute gouty arthritis, in Talbott's experience.

ports have indicated that alterations in adrenocortical function may be important in the mechanism of acute gouty arthritis (33, 37, 43-47). However, there is no clear evidence to indicate that adrenocortical hyperfunction is continuously present in interval gout. One example may be cited. In spontaneous adrenocortical hyperfunction of the Cushing type, and in certain stress situations, differential leukocyte counts show marked eosinopenia and lymphopenia. Similar changes may be produced in man by the administration of the purified adrenocorticotrophic hormone or by certain metabolic steroids of the adrenal cortex. Table 5 shows that eosinopenia and lymphopenia are not present in interval gout.

Robinson, Conn, Block and Louis (45) have recently presented convincing evidence of the absence of chronic "resistance stage" status in one carefully studied gout patient to whom adrenocorticotropin was administered. Before the hormone was administered, the patient, then in a prolonged interval phase, was found to have a normal output of 11-oxysteroids (measured as formaldehydogenic steroids). ACTH was found to elicit a normal increase in 17-ketosteroid excretion. Patients in the "resistance stage" show both an increase in basal 11-oxysteroid output and a substandard increase in 17-ketosteroid output under stress conditions, unlike this gout patient. We have recently confirmed these findings in 2 additional gout patients (48).

Elimination of the general adaptation syndrome exhausted the established causes of very low 17-ketosteroid output. It was then necessary to see whether the hypogonadism, expected to occur with low 17-ketosteroid outputs, actually did occur in gout. In only one of the patients studied was evidence of hypogonadism found. In the others biologic androgen activity appeared normal.

Table 7 summarizes observations on a larger group of gouty men. It will be noted that almost all gout patients marry, and that the number with children and the number of children per patient are typical of normal adults. Six of the 27 patients with children sired one or more after the onset of clinical gout, which occurred at an average age of 41 years. This is unusually late, and suggests that some patients forgot their initial attack. It may well be, therefore, that the number of patients siring children after the initial attack was actually considerably greater. Our 2 patients with six and seven children respectively both reported the onset of clinical gout to have preceded the birth of *all* children. Since normal gonadal function certainly existed when clinical gout was present in these patients, the findings imply that it may exist when decreased ketosteroid excretion is present.³

³ One patient (H.N.) noted exacerbations of facial acne 24 to 72 hours before the onset of gout attacks. Similar exacerbations of acne may be produced by the administra-

served to make "retention" of 17-ketosteroids improbable. In spite of accumulating evidence that altered adrenocortical function may be important in attacks of gout, there is no evidence of "resistance stage" endocrine status during interval gout.

Biologic evidence of androgen activity was normal in 9 of the 10 men in this group. A review of a much larger series of patients gave additional evidence that hypogonadism is not clinically prominent in patients with gout.

The finding of very low outputs of urinary 17-ketosteroids in the presence of normal biologic androgen activity appears to be a new endocrine finding which is characteristic for gout. Our working hypothesis assumes that, in gout, biologic androgen activity is maintained by an androgenic hormone which does not make an important contribution to urinary 17-ketosteroids when metabolized.

Acknowledgment

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tions (Table 2 and (39, 40)), but this is not surprising in a group of men who average 45 years of age when first seen by the physician. Detailed reasons for rejecting the established causes of diminished 17-ketosteroid outputs as possible explanations have been given. Previous investigators have described only two conditions, both in females, in which an abnormal increase in biologic androgen activity was not accompanied by a proportionate increase in 17-ketosteroid excretion. In arrhenoblastoma (50), 17-ketosteroid excretion may be normal or low when marked masculinization is present, and ketosteroid excretion with such tumors rarely is comparable to that seen in virilization due to adrenocortical hyperplasia or tumor. In simple hirsutism in women, 17-ketosteroids were subnormal in approximately one-third of the patients investigated by Bissell and Williams (51). Unfortunately, hirsutism as a solitary sign is not well established as purely androgenic in origin. There appear to be no reports of conditions in which normal androgenic activity accompanies low ketosteroid outputs.

The concurrence of very low 17-ketosteroid outputs with normal biologic androgen activity in gout appears to be a new endocrine finding. We believe the diminished ketosteroid output to be referable to decreased production of normal precursors because exogenous testosterone, the presumptive chief active normal gonadal hormone (39), was normally converted to ketosteroid. The finding of normal biologic androgen activity means that the gouty must produce some type of androgen. The *working hypothesis* adopted postulates that, in gout, biologic androgen activity is maintained by an androgen which does not make an important contribution to urinary 17-ketosteroids when metabolized. Since hyperuricemia may be inherited by either sex (3, 4, 5, 6, 30, 31, 34) and since clinical gout occurs in both men and women, such an abnormal androgen would presumably originate in the adrenal cortex. The finding of such a substance might well clarify the complex metabolic problems implicit in the observations listed in Table 1.

CONCLUSION

Decreased urinary 17-ketosteroid output has been found in all of a group of 11 gout patients. It was present in all stages of the disease, including asymptomatic interval gout. A similar decrease was not found in a small group of patients with idiopathic hyperuricemia, nor was it present in males with rheumatoid arthritis or spondylitis.

A review of the currently available endocrine explanations for decreased 17-ketosteroid output of the degree found indicated none to be acceptable. Injected testosterone was recovered as urinary 17-ketosteroid to the usual extent and no defects in hepatic function were found with a representative group of sensitive liver function tests. Renal function was well enough pre-

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PREGNANCY IN ADDISON'S DISEASE

REPORT OF FOUR PATIENTS

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INTRODUCTION

THE occurrence of pregnancy in patients with Addison's disease presents certain features of physiologic interest and poses several problems in therapy. Most standard textbooks state that the maternal adrenals enlarge in pregnancy, though their contribution to the normal progress of gestation remains for the most part unknown. An approach to this problem has been made by Venning by determining urinary excretion of substances derived from the adrenal in 9 normal pregnant women (1). In the first trimester of pregnancy a temporary increase was observed in the excretion of glycogenic corticoids to more than double that found in normal non-pregnant women, but early in the second trimester, there was a return to the values found before pregnancy. A second and greater rise to 5 times these values was noted in the latter part of pregnancy, with finally a fall before delivery. The 17-ketosteroid excretion increased gradually throughout pregnancy when assayed with m-dinitrobenzene (Zimmermann reaction). This increase was interpreted by Venning as due to an increase in 20-ketosteroids rather than in 17-ketosteroids, since the latter as determined with antimony trichloride (Pincus method) remained unaltered.

Certainly the presence of the adrenal glands is not essential either for the establishment of pregnancy or for its maintenance. There are available 29 reports of 39 pregnancies in women with Addison's disease, 30 of which continued to term (2-29). Although in certain of these reports the data given are insufficient to establish the diagnosis of adrenal insufficiency, in the great majority the records are of unquestionable authenticity. In only one such patient has a study of the steroid excretion been reported (26). In this patient, Samuels and associates found that the excretion of estrogens, pregnanediol and of 17-ketosteroids (assayed by a modification of the Zimmermann reaction) during the last two trimesters corresponded to the amounts excreted in normal pregnancy. An assay of the corticoid excretion was not made.

Reports concerning gonadal function in adrenalectomized animals have been conflicting; however, it appears definite that estrous cycles continue

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24-hour urine specimens were made by the the method of Jailer (37); gonadotropins by the method of Levin and Tyndale (38); sodium pregnanediol glucuronide¹ by the technique described by Venning (39); corticoid by the glycogen deposition test of Venning, Hoffman and Browne (40);² and 17-ketosteroids, for the most part, by the Pincus reaction (41), although a few values were obtained by the Zimmermann reaction (42).

CASE HISTORIES

Case 1, P. H. #627658, E. G. was a 32-year old white housewife who was admitted in the seventh week of her first pregnancy. The diagnosis of Addison's disease had been established seven years previously, at which time she had an eleven-month history of increasing pigmentation of the skin and a four-month history of nausea, vomiting, and weight loss of 10 pounds. There was no background of tuberculosis. On physical examination increased pigmentation was evident although it did not extend to the mucous membranes. Her blood pressure was 95/65. Laboratory studies at that time revealed the following values: serum sodium, 125 mEq. per liter; serum potassium, 5.5 mEq. per liter, fasting blood sugar, 80 mg. per cent; urinary excretion of 17-ketosteroids (Zimmermann reaction), 2.9 mg. in 24 hours; and basal metabolic rate, minus 17 per cent. A roentgenogram showed that the chest was normal and an abdominal film showed no evidence of adrenal calcification. A tuberculin test, 0.1 cc. of O.T. in a 1:100,000 dilution, gave negative results. With the administration of 3 mg. of desoxycorticosterone acetate (DCA) daily by subcutaneous injection she remained in fairly good health for the ensuing seven years, with the exception of one admission in a crisis precipitated by iritis. At this time the value for serum sodium was 110 mEq. per liter and for blood sugar, 45 mg. per cent.

At the time of admission for pregnancy, a dilatation and curettage was advised. Beginning the day before the contemplated procedure she received whole adrenal extract (ACE) intramuscularly in addition to the maintenance dose of DCA, 5 cc. the day before operation, 10 cc. on the day of operation and 10 cc. the day after. The ACE used was the hog adrenal lipo-cortex of Upjohn, 1 cc. of which is the equivalent of 4 mg. of corticosterone or of 2 mg. of Compound E. Scopolamine and demerol were used as preoperative medication, and an infusion of glucose and saline was started before moving the patient to the operating room. Cyclopropane was selected as the anesthetic agent. Dilatation and curettage was performed by Dr. D. A. D'Esopo, the operation lasting ten minutes and the anesthesia, twenty minutes. In spite of this short procedure with negligible blood loss, upon return to the ward, the patient went into a state of shock: the pulse became feeble, the blood pressure fell to 82/40 and temperature to 96°. Response to shock therapy and transfusion, however, was prompt and her subsequent course uneventful.

One year later this patient died at the Norwalk hospital following an upper respiratory infection. Dr. L. A. Giuliano has kindly informed us that an autopsy performed by Dr. R. N. Barnett showed extreme atrophy of the adrenals.

Case 2, P. H. #811755, M. S. was a 31-year old housewife admitted in the fourth month of her second pregnancy. This patient's first pregnancy has been reported by Sheldon (27). At that time Addison's disease was suspected from the characteristic pigmentation of the skin and mucous membranes and a blood pressure of 100/70, and the

¹ These determinations were very kindly made by Dr. L. Levin.

² We wish to thank Dr. E. H. Venning for performing the determinations.

in such animals if they are maintained in good condition by the ingestion of salt, and that pregnancy may occur (30, 31). It has also been claimed that pregnancy and pseudopregnancy may even prolong the survival time of untreated adrenalectomized animals (32, 33). In addition, postpartum lactation has been observed in adrenalectomized rats maintained in good condition with sodium chloride added to the diet (34).

Despite the fact the pregnancy may occur and progress to term in the absence of adrenal cortical function, it remains a serious complication for the patient with Addison's disease. By far the majority of authors have observed that the health of such patients deteriorates during gestation, and in 8 of the 12 well documented reports, death of the mother has resulted during the course of pregnancy, immediately post-partum or within a short period thereafter. From a consideration of the normal physiologic changes in pregnancy, difficulties peculiar to the patient with Addison's disease might be expected. The nausea and vomiting in the first trimester, with accompanying disturbance in electrolyte balance, might not be well tolerated. In the last trimester, on the other hand, the increased blood volume and the possible contribution of hormones from the fetal adrenals might have a favorable effect upon the adrenal insufficiency. However, serious difficulties might be anticipated at the time of delivery as a result of blood loss, and again in the postpartum period during the diuresis which follows the withdrawal of placental progesterone. Finally, in the patient whose adrenal disease is on a tuberculous basis, there is a possibility of activation of the tuberculosis during pregnancy.

It is to be expected that the problem of pregnancy in Addison's disease will become increasingly frequent, since the life expectancy and general well being of these patients has been substantially increased with recent advances in therapy. It seems worthwhile, therefore, to report the findings in 4 such patients observed at the Presbyterian Hospital.

METHODS

Four patients with documented Addison's disease were studied during 5 pregnancies. One pregnancy ended in a spontaneous abortion, a second was terminated by dilatation and curettage and a third by hysterectomy. In 2 of the patients the pregnancy went to term. One of these was delivered by Caesarian section, and the other had a spontaneous delivery.

Serum sodium concentration was calculated by summation of the chloride values and carbon dioxide content expressed as milliequivalents plus 10, or was determined directly by use of an improved type of internal standard flame photometer with an accuracy of ± 1 per cent (35): potassium was determined after precipitation as chloroplatinate (36) or directly with the flame photometer. Determinations of estrone plus estradiol in

generalized pigmentation of the skin in addition to brown patches on the buccal mucous membranes. Her blood pressure was 92/60. The level of her serum sodium on admission was 132 mEq. per liter, and her fasting blood sugar 81 mg. per cent. The urinary excretion of 17-ketosteroids determined subsequent to pregnancy was less than 1 mg. in 24 hours. X-ray examination of the chest and of the adrenal area revealed no evidence of tuberculosis. A tuberculin test with 0.1 cc. of O.T. carried to a dilution of 1:100, gave negative results.

Therapy was initially directed at controlling her adrenal insufficiency with the hope that, as she entered the third trimester, the increased blood volume of this period would result in an improvement in her condition. However, this did not prove to be the case, and although her serum sodium values were soon raised to normal levels with DCA and added sodium chloride, her condition continued to degenerate, she developed multiple complications and became progressively debilitated, losing an additional 5 kilograms of

TABLE 1. ADDISON'S DISEASE. CASE 3. PATIENT H.R.: CLINICAL COURSE AND THERAPY DURING PREGNANCY

Month of gesta- tion	Weight, Kg.	Blood pressure, mm. Hg	Serum sodium, mEq./L.	Blood sugar, mg. %	DCA, mg./24 hrs. s.c.	NaCl (Gm./24 hrs.)		Remarks
						Parenterally	Orally	
5	49	88 — 40	131 138	81	5	2*	3	↑ nausea and agranulocytosis; jaundice ↓ vomiting
6		92 — 60	138		6	2.1*	4	
7	48	110 — 68	137	59	6	6.2*	4	
8		128 — 84	139		6	4.9*	4	
9	43	154 — 90	137		6	3*	2	

Labor 32 days after expected date of delivery.
* Average daily NaCl supplied by infusion, transfusion or plasma.

weight. In Table 1 her clinical course, laboratory findings and therapy are outlined month by month during pregnancy, and in Table 2, day by day, during and following delivery.

Five major problems arose during pregnancy. The first was the control of her adrenal disease. Nausea and vomiting persisted to term and necessitated frequent infusions of glucose and saline (total of 60 liters) to maintain an adequate intake of sodium and carbohydrate. Secondly, with the hemodilution attendant on the correction of the adrenal insufficiency, a severe anemia and hypoproteinemia became evident (rbc 2.4 million and serum protein 4.5 Gm. per cent). These persisted to term in spite of repeated transfusions (total of 7.5 liters of blood). Thirdly, two months after admission she developed a transient agranulocytosis (wbc 1100, polymorphonuclears 1 per cent) presumably from sulfadiazine which had been administered in small doses prophylactically for a mild right hydronephrosis. After discontinuation of the drug, her white blood cell

diagnosis was established by salt withdrawal in a Cutler-Power-Wilder test. X-ray examination of the chest and of the adrenal area showed them to be normal at that time and also subsequently. A tuberculin test reaction was reported as negative, whereas in 1946, 0.1 cc. of O.T. in a 1:10,000 dilution gave a mildly positive reaction. There was no family history of tuberculosis. The postpartum course following her first pregnancy was reported as stormy, with high fever and blood pressure at shock level for seven days after delivery. Her general condition in the intervening years had deteriorated and with the onset of the second pregnancy there had been increasing weakness. An intercurrent respiratory infection led to admission to an outside hospital, where severe nausea and vomiting resulted in a weight loss of 6 kilograms and necessitated repeated infusions. Because of her poor condition and known previous difficulties following delivery, termination of this pregnancy was considered advisable.

Beginning the day before operation, she was given ACE intramuscularly in addition to her maintenance dose of DCA. The same preoperative medication and anesthesia were employed as in Case 1. Dr. H. C. Taylor performed a supravaginal hysterectomy. The operative procedure lasted fifty minutes, during which time a transfusion was given. The patient withstood the operation well. During the next two days, however, she became gravely ill, presenting the picture of acute adrenal insufficiency. Her blood pressure remained alarmingly low (on one occasion 75/50), serum sodium concentration fell to a minimum of 127 mEq. per liter and her temperature rose to over 103° without clinical or laboratory evidence of infection. This condition developed in spite of treatment with up to 20 cc. of ACE daily, maintenance doses of DCA, a daily oral and parenteral intake of 10-20 Gm. of sodium chloride, and supportive measures in the form of blood (1000 cc. on the day of operation, 350 cc. on each of the two succeeding days), as well as plasma and glucose infusions, antibiotics and chemotherapy.

The parenteral carbohydrate given the day of operation was inadequate to compensate for the postoperative reduction in food intake and the morning following operation her blood sugar level was 54 mg. per cent. Subsequently hypoglycemia was prevented with frequent parenteral or oral carbohydrate feedings. The one further complication which appeared was a fall in serum potassium content to a minimum of 2.4 mEq. per liter. This was observed on the fourth postoperative day after large quantities of adrenal extract had been administered in combination with considerable amounts of parenteral fluid which, apart from transfusions, contained sodium as the only cation. Although the patient showed no abnormality of neuromuscular function, 1.5 Gm. of potassium chloride was administered by mouth with prompt return of the serum potassium level to normal. It must be pointed out that without repeated determinations of serum potassium concentrations, the administration of potassium might have been hazardous.

The patient's recovery was subsequently uneventful and one year later her condition was satisfactory. However, at the time of writing, information has just been received of her death in an another hospital. Autopsy findings included lobar pneumonia and atrophy of the adrenals.

Case 3, P. H. #813457, H. R. was a 36-year old white housewife in whom the signs and symptoms of Addison's disease appeared coincidently with her first pregnancy. She was initially seen and admitted in the fifth month of gestation at which time she gave a history of six months of increasing pigmentation. Nausea and vomiting dated from the early weeks of pregnancy but persisted and became increasingly severe and were associated with a weight loss of 11 kilograms. There was no history of tuberculosis and her health prior to pregnancy had been good except for chronic sinusitis. On physical examination she was emaciated, appeared chronically and severely ill and there was deep

count gradually returned to normal. Fourthly, shortly after the episode of agranulocytosis she became jaundiced. Homologous serum hepatitis was suspected, but the findings of intermittently clay colored stools, a negative result with the cephalin flocculation test and a bilirubinemia of 5 mg. per cent, were more suggestive of obstruction. The serum phosphatase values rose to a peak of 9.6 Bodansky units, but, in view of the normal elevation in pregnancy (43), this could not be considered as additional evidence in favor of obstruction. The jaundice persisted until delivery, then subsequently cleared. Fifthly, in the month before delivery she showed a moderate hypertension. This appeared to be specifically related to pregnancy since the blood pressure returned to normal following delivery and no elevated readings have been obtained in the subsequent three years of observation.

The patient went into labor thirty-two days after the expected date of delivery. With the onset of labor a slow infusion of saline was started and ACE was substituted for DCA. At the end of ten hours there had been no progress in labor and the cervix remained dilated only one finger breadth. An operative delivery was decided upon and under cyclopropane anesthesia Dr. D. A. D'Esopo performed a classical Caesarian section. The patient withstood the procedure well, maintaining the hypertension throughout. A liter of blood was given during and immediately after the operation. The baby was a 2400 Gm. male infant of 50 cm. length, who died twenty-four hours later from unexplained mediastinal emphysema. The adrenals weighed 11 Gm. (the normal values for infants of this age and size range from 2.8 to 11.0 Gm., according to Benner (44)). There were no other abnormalities.

The day following delivery a second operation was necessitated because of oozing at the operative site and again the patient withstood the procedure well. However her subsequent postpartum course was stormy. As in the case of the previous patient, this woman became acutely ill over a three-day period following delivery, with high fever for which no infectious basis was determined. There was a drop in serum sodium concentration to a minimum of 130 mEq./per liter, although at no time did her blood pressure approach hypotensive levels. In addition to the therapy outlined in Table 2, she was given penicillin and streptomycin. Her temperature gradually returned to normal and her clinical condition improved. The fifth and sixth day after delivery, engorgement of the breasts was noted.

The patient has been observed for three years since delivery. Her general health remains poor but without any residual findings definitely related to pregnancy.

Case 4, P. H. 814847, S. R. was a 24-year old white housewife with known Addison's disease, admitted for vaginal bleeding in the eighth week of her first pregnancy. A diagnosis of adrenal insufficiency had been made eight months previously at the Mt. Sinai hospital, at which time she gave a history of increasing pigmentation and asthenia which had progressed alarmingly following an appendectomy two months before her admission to Mt. Sinai. There was no history or x-ray evidence of tuberculosis. Control of her hypoadrenalism was effected with a daily dose of 1.5 mg. of DCA subcutaneously and 4 Gm. of enteric coated sodium chloride orally.

At the time of her admission to Presbyterian Hospital she showed evidence of generalized pigmentation of the skin with many black freckles and several gray brown spots on the lips, gums, tongue and buccal mucous membranes. Her blood pressure was 100/70. There was a brownish vaginal discharge but the cervix appeared to be closed. Laboratory studies revealed values for serum sodium of 139 mEq. per liter and for fasting blood sugar of 69 mg. per cent. Urinary 17-keto-steroid excretion as determined in the non-pregnant state was less than 1 mg. in 24 hours. X-ray examination of the chest and adrenal area revealed nothing abnormal.

TABLE 2. ADDISON'S DISEASE. CASE 3. PATIENT H.R.: CLINICAL COURSE AND THERAPY DURING LABOR AND PUERPERIUM

Day	Temp.	Blood pressure, mm. Hg.	Serum sodium, mEq./L.	Weight, Kg.	DCA, mg. s.c.	ACE*, cc. i.m.	NaCl, Gm.f	Parenteral fluids		
								Blood cc.	Plasma cc.	Glucose + saline cc.
Day before labor	98.8°	160 — 80		43.0	6		2			
Labor and Caesarean section	99.2°	168 — 98	135			10	18	1000		1000
1 Second operation	100.8°	138 — 80				15	18	1500		500
2	100.4°	142 — 80				15	14	500	500	
3	101.2°	150 — 90	132		5	20	9		500	
4	101.8°	152 — 88	130		5	5	5			500
5	102.4°	154 — 70	135		5		5			
6	103.4°	152 — 92			5		15		500	
7	103.4°	130 — 80			5		10			1000
8	102.6°	130 — 80			5		14			1000
9	102.2°	128 — 84			5		6			
10	100.6°	130 — 78	138		5		6			
Day of discharge		108 — 80	140	41.5	2		7			

* Upjohn's lipo-adrenal cortex.

† Sum of parenteral and oral administration.

TABLE 4. ADDISON'S DISEASE. CASE 4. PATIENT S.R.: CLINICAL COURSE AND THERAPY DURING LABOR AND PUERPERIUM

Day	Temp.	Blood pressure, mm. Hg	Serum sodium, mEq./L.	Weight, Kg.	DCA, mg. s.c.	ACE*, cc. i.m.	NaCl†, Gm.	Parenteral fluids	
								Blood cc.	Glucose +saline cc.
Day before labor	99.4°	112 — 74	135	60.3	4		4		
labor	98.6°	124 — 80	137		4	10	22	500	2000
1	100.2°	104 — 70	135			5	10		700
2	99°	102 — 58			4		5		
3	99.8°	104 — 70	134		4		5		
4	99°	106 — 74			4		5		
Day of discharge		108 — 76	144	54.1	3		5		

* Upjohn's lipo-adrenal cortex.

† Sum of parenteral and oral administration.

During the seventh month an intercurrent bronchitis precipitated an Addisonian crisis which responded to penicillin, parenteral fluids and ACE given in addition to maintenance doses of DCA.

Symptoms of hypoglycemia recurred frequently and were documented on many occasions with a decrease in blood sugar levels, at one time to as low as 35 mg. per cent. This problem was fairly well controlled by the use of between-meal and midnight feedings.

The patient went into labor twenty-three days after the expected date of delivery. ACE therapy and a slow infusion of glucose and saline were then begun. Over the course of the next seventeen hours she received a total of 2000 cc. of fluid parenterally, and her condition remained excellent. At the end of this time the patient was delivered under cyclopropane anesthesia, by Dr. D. A. D'Esopo, of a normal female infant weighing 2870 Gm. Following delivery a transfusion of 500 cc. was given. The postpartum course of the patient was entirely uneventful and she was ambulant on the fifth day. The patient's breasts were engorged three days post-partum. No attempt was made at breast feeding and seventeen days post-partum her breasts were soft with only a slight watery discharge.

She has been observed over two ensuing years and her condition remains satisfactory. The baby has been well. At the time of writing, she is again pregnant.

TABLE 3. ADDISON'S DISEASE. CASE 4. PATIENT S.R.: CLINICAL COURSE AND THERAPY DURING PREGNANCY

Month of gestation	Weight, Kg.	Blood pressure, mm. Hg	Serum sodium, mEq. L.	Blood sugar, mg. %	DCA, mg., 24 hrs. s.c.	NaCl (Gm./24 hrs.)		ACE, cc. i m.	Remarks
						Parenterally	Orally		
2		110 — 70	128 138	76	376 mg. in pellets implanted 4 mo. before pregnancy		6		nausea vomiting
3	52.2	95 — 58	135	55			6		recurrent hypoglycemia
4		94 — 68	135	35			6		
5		86 — 50	137	63			6		
6	54.5	98 — 56	138	53			4		
7	56.4	100 — 60	134	67	3	53*	4	30*	
8		98 — 70	135		3		4		
9	60.3	112 — 70	134	76	3		4		

Labor 23 days after expected date of delivery.

* Given during a 3-day illness; acute bronchitis.

† Upjohn's lipo-adrenal cortex.

On her third hospital day the patient had a spontaneous complete abortion, with no exacerbation of her adrenal disease.

The patient was discharged and two months later she had 376 mg. of DCA in pellet form implanted at the Mt. Sinai Hospital. Four months after this she again became pregnant. In contrast to Case 3, her condition during pregnancy remained on the whole very satisfactory and she gained a total of 8 kilograms. During this pregnancy she was admitted to the hospital on 4 occasions for observation or for intercurrent infection. She remained in the hospital during the ten weeks prior to delivery. Her course, laboratory findings and therapy month by month during pregnancy are outlined in Table 3, and day by day following delivery, in Table 4.

Control of her adrenal disease during the early months of pregnancy was effected by the implanted DCA pellets and oral administration of 6 Gm. of enteric coated sodium chloride daily. With the nausea and vomiting of the first trimester, she had on one occasion a fall in the level of serum sodium to 128 mEq. per liter, but subsequent determinations were within the normal range. After absorption of the pellets, DCA was given by injection in doses of 3 mg. daily and the sodium chloride was reduced to 3 Gm. per day.

in pregnancy, in which levels as high as 750–1000 micrograms have been obtained at term with the fluorometric method used (Jailer, unpublished data). The value for sodium pregnanediol glucuronidate was normal in H.R. (Case 3); the several determinations on S.R. (Case 4) were somewhat low. In the one patient in whom the determination was made, there was no corticoid activity demonstrable in the urine on two occasions. The 17-ketosteroid excretion in S.R. (Case 4) rose during pregnancy to a peak of 7.4 mg., but fell to 3.7 mg. five days before delivery. In H.R. (Case 3) the one determination of 17-ketosteroids made during pregnancy was significantly greater than the determination one year later.

DISCUSSION

It is impossible to draw many conclusions about pregnancy in Addison's disease based on so small a group of patients. However, certain points bear discussion.

Examination of the data on urinary hormone excretion indicates that those substances derived from the placenta, *i.e.* gonadotropins, progesterone (sodium pregnanediol glucuronidate), estrones and estradiol, were excreted in normal or but slightly decreased amount by these patients with hypoadrenalism. From this observation it would appear that a normal maternal adrenal cortex is not necessary for the elaboration by the placenta of sufficient amounts of these hormones to maintain pregnancy.

The absence of corticoid activity in the urine of the one patient (S.R., Case 4) in whom it was assayed, is of interest. Two determinations were made late in pregnancy at which time greatly increased activity is indicated by the values found in normal urine. This normal increase in corticoid excretion late in pregnancy was first thought to reflect excretion from the fetal adrenals, which enlarge strikingly at this time. However, both Venning (1) and Day (45) have found the urine of new born infants to show little or no corticoid excretion. The failure to demonstrate such activity in the urine of pregnant patient S.R. with Addison's disease, offers support to Venning's concept that the increased amounts of corticoid-like material, found normally in pregnancy urine, reflect increased function of the maternal adrenal cortex.

The observed increase in 17-ketosteroid excretion raises a question as to the source of this excretory product in these patients with adrenal insufficiency. Admittedly, in the case of H.R. (Case 3) only one determination was made during pregnancy, and although this value was significantly greater than the one obtained one year later, this could be no more than a reflection of progressive adrenal degeneration. However, in S.R. (Case 4) the several determinations during pregnancy indicate a gradual increase in secretion with a subsequent fall, and Samuels, Evans and McKelvey

Urinary excretion of hormones

The determinations of urinary excretion of hormonal substances are shown in Table 5. It is evident that the data are limited. The gonadotropic hormones, in the 3 patients in whom these were estimated, were excreted in amounts comparable to those observed in normal pregnancy. The estrogen excretion in M.S. (Case 2) was not abnormal, but in E.G. (Case 1) and S.R. (Case 4) the values were somewhat lower than those usually found

TABLE 5. URINARY EXCRETION OF HORMONES IN 4 PREGNANT WOMEN WITH ADDISON'S DISEASE

Patient	Month of gestation	Gonadotropins, mouse units	Estrone plus estradiol, μ g./24 hrs.	Sodium pregnanediol glucuronide, mg./24 hrs.	17-Ketosteroids, mg./24 hrs	Corticoids, glycogen units
S.R. (Case 4)	2	180,000		8.9		
	5	14,000	326	10.5 1.6	1.8	
	7		89	14.5	1.6 2.7	
	8		320 374		5.7	0
	9		287 442		7.4 3.7	0
	1 year later				less than 1*	
H.R. (Case 3)	7				4.5*	
	8	3,000		70		
	1 year later				less than 1*	
M.S. (Case 2)	4	12,000	94		2	
E.G. (Case 1)	2		30		1.5	

* Determined by the Zimmermann reaction.

course was unexpectedly benign. Finally it is of interest that lactation occurred in both these women, although its adequacy was not tested by breast feeding.

Of the 2 patients in whom pregnancy was terminated early by operation, one (M.S. Case 2) exhibited a classical Addisonian crisis following hysterectomy. Such a reaction did not occur in E. G. (Case 1) after dilatation and curettage; however, the short episode of shock may have been related to the known vasomotor instability of patients with adrenal insufficiency.

SUMMARY AND CONCLUSIONS

1. Five pregnancies were observed in 4 patients with Addison's disease. All patients survived. One pregnancy ended in a spontaneous abortion, 2 were terminated operatively early in pregnancy, and 2 went to term.

2. In these patients, urinary excretion of gonadotropin was normal. The values obtained for sodium pregnanediol glucuronidate and estrogens, though low, were within the range seen in normal pregnancies. Corticoid activity could not be demonstrated in the urine of 1 patient studied in the third trimester. The urinary excretion of 17-ketosteroids was increased late in pregnancy in the 2 patients in whom gestation proceeded to term.

3. The clinical management of pregnancy complicated by Addison's disease is briefly discussed.

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(26) have reported an even more striking rise in another such patient. These workers suggested that this rise was due to secretion by the fetal adrenal. However both Talbot and co-workers (46) and Day (45) have reported low 17-ketosteroid excretions in new born infants. Day found values ranging from 0.13 to 3.25 mg. in the 24-hour excretion of 12 such cases, from which it appears unlikely that the fetal adrenal contributes significantly to the increased excretion in pregnancy. Hence, the increased 17-ketosteroid excretion observed in pregnant women with adrenal insufficiency suggests that this excretory product may be derived from some source other than the adrenal. One possible, though purely speculative, interpretation is that the placenta contributes to the observed rise.

Clinical generalizations concerning the management of pregnancy in Addison's disease must be limited, since in only 3 of the patients reported here did gestation continue long enough to permit observations as to its influence upon the underlying adrenal disease. Furthermore the course of these patients was variable—the condition of two, M.S. (Case 2) and H.R. (Case 3) unquestionably deteriorated during pregnancy whereas the general health of the third, S.R. (Case 4), remained excellent. Of the anticipated difficulties, the nausea and vomiting of early pregnancy did not present a serious problem for S.R., although on one occasion a significant fall in the value for serum sodium resulted. On the other hand, in M.S., vomiting necessitated electrolyte replacement with saline infusions and in H.R. it continued to be a most serious hazard throughout pregnancy. Furthermore, H. R. failed to obtain any relief in symptoms, as anticipated, upon entering the third trimester and because of the persistent vomiting, larger amounts of sodium chloride were required to maintain adequate electrolyte balance during gestation than were subsequently needed. However, the daily dosage of DCA was continued unaltered. In S.R. the maintenance therapy with both DCA and sodium chloride did not require alteration as a result of pregnancy.

At the termination of each pregnancy, therapy consisted of the administration of DCA by injection and sodium chloride by mouth; and in addition, parenteral saline, glucose, plasma and whole blood as indicated. Each patient also received hog adrenal lipo-cortex (ACE). This extract has a definite effect on salt and water metabolism and the clinical studies of MacBryde and de la Balze (47), show an effect on carbohydrate. Observations in this hospital have not confirmed an effect on carbohydrate metabolism in humans with doses up to 15 cc. per day (unpublished data); however, its value in Addisonian crises has clinically seemed superior to that of desoxycorticosterone acetate (DCA).

Following delivery, increased manifestations of adrenal insufficiency occurred as predicted in H.R. (Case 3), whereas S.R.'s (Case 4), postpartum

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PHYSIOLOGIC	INDEX	CHANGES FOLLOWING 25 mgm. DOSE OF ACTH	CHANGES FOLLOWING REPEATED DOSES
ADRENAL CORTEX (in rats)			
ASCORBIC ACID CONTENT		↓ ³	↑ ³
CHOLESTEROL CONTENT		↓ ³	↑ ³
BLOOD (in human beings)			
EOSINOPHILIC LEUCOCYTES		↓	↓
LYMPHOCYTES		↓	↓ OR ○ ¹
HEMOGLOBIN CONCENTRATION AND HEMATOCRIT		○	↓
BLOOD SUGAR			↑ OR ○ ¹
SERUM CO ₂ COMBINING POWER			↑
SERUM INORGANIC PHOSPHORUS			↓
SERUM URIC ACID		○	○
SERUM PROTEIN AND ELECTROPHORETIC PATTERN		○	○
FREE PLASMA CHOLESTEROL			↓ ¹
URINE (in human beings)			
17-KETOSTEROIDS		○ ¹ (↑ AFTER 100mgm.) ¹	↑
11-OXYSTERIODS		↑ ¹	↑ ¹
VOLUME			↓
URIC ACID		↑	↑
CREATININE		○	○ OR ↓
URIC ACID-CREATININE RATIO		↑	↑
CREATINE			↓ ¹
SODIUM		↑	↓ OR ○ ¹
CHLORIDE		↑	↓ OR ○ ¹
POTASSIUM		↑	↑ OR ○ ¹
PHOSPHORUS			↑ OR ○ ¹
CHOLESTEROL			↓ ¹
TOTAL NITROGEN			↑ OR ○
GLUCOSE			↑ ² OR ○ ¹
SKIN (in human beings)			
ACNE			↑ ¹

Data from Forsham et al (1948) except where otherwise indicated.
¹ Reported by Mason et al (1948)
² Reported by Conn et al (1948)
³ Reported by Sayers et al (1945)

FIG. 1. Physiologic changes following administration of anterior pituitary adrenocorticotrophic hormone in animals and human beings.

Other workers regard the 17-ketosteroids as an unreliable means of determining adrenal activity (23) since they find variable 17-ketosteroid values in both physiologic and pathologic stresses. It has been shown that the urinary glyco-genic corticoids (11-oxysteroids) may be rising while the 17-ketosteroids are falling in a stress situation (24). Until more reliable

HORMONAL ALTERATIONS IN MEN EXPOSED TO HEAT AND COLD STRESS

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THE adrenal cortex plays a role in the body's defense against cold (1, 2, 3, 4), but the precise nature of this role has not been clarified. Bilateral destruction of the adrenal gland diminishes resistance to varying types of stress including muscular exercise, burns, infections, shock, trauma, anoxia and cold (2). Histologic studies reveal adrenal hypertrophy (5, 6, 7), and chemical studies show a diminution in the cholesterol and ascorbic acid content of the adrenal cortex in rats exposed to cold (8, 9). In fact, so well established is the relationship between the adrenal cortex and the stimulus of cold, that the latter is used as a standard stress in the biologic assay of adrenal cortical extracts (10).

Indirect evidence that outpouring of the adrenal cortical hormones is responsible for the changes seen in the "general adaptation syndrome" (11) is provided by experimental administration of adrenal cortical extracts (12). There is involution of thymus and lymphoid tissue and fall in circulating lymphocytes following injection of adrenal cortical extracts. The lymphocyte fall has been used as a criterion for adrenal response to stress in human beings (13, 14, 15).

The urinary excretion of 17-ketosteroids has been regarded as an index, to some extent, of the rate of adrenal cortical hormone excretion (16, 17). Some workers found a decreased output with a slow return to normal following trauma, bacterial infections and surgical procedures (16). Others (18, 19) found that in normal individuals, the excretion of 17-ketosteroids usually rose for twelve to forty-eight hours, then fell to a subnormal rate and gradually returned to normal. A sharp rise in 17-ketosteroid excretion immediately after stress with an abrupt fall below normal was also found in another study on 30 patients who had experienced hemorrhage, fracture, burn, infection, or operation (20). Elevated excretion of 17-ketosteroids has been reported in muscular fatigue (21), aircraft pilots (22) and in subjects exposed to cold for two hours (17).

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posure to cold, with a coincident rise in basal metabolic rate. Rats exposed to cold previous to thyroidectomy survive longer in cold after thyroidectomy than thyroidectomized rats not previously exposed (30). This finding, plus the fact that increased thyroid activity occurs only after an initial period of normal or low basal metabolism in human beings and animals, suggest other mechanisms, which apparently adequately defend the organisms during the early periods of cold exposure.

On the basis of the above evidence, an investigation of adrenal and thyroid responses of men subjected to extreme heat and cold was undertaken. The observations were carried out simultaneously with studies of acclimatization to heat and cold. (41).

METHODS

Experimental Design

Three healthy, white males were exposed successively to a preliminary two-week period of physical conditioning; to 19 five and one-quarter hour periods of heat (107°F., dry bulb; 89° F. wet bulb; wind 3 m.p.h.); to 14 five-hour periods of cold (−20°F., wind 3 to 4 m.p.h.); to 5 re-exposures to heat; a five-week interval of no exposure to environmental stress; and finally to 3 re-exposures to heat.

Throughout these periods, studies were made on the circulating eosinophils, absolute numbers of lymphocytes, urinary uric acid-creatinine ratio, urinary 17-ketosteroids, and basal metabolism. In addition, in order to evaluate acclimatization, cardiovascular responses, sweating loss, metabolic rates on treadmills, blood volume, thiocyanate space, water and salt balances, skin and rectal temperatures were measured and are reported elsewhere (41).

Food and fluid intake for each test subject was recorded on a daily basis beginning with the evening meal. No effort was made to control the quantity of food or fluid consumed by the test subjects except that no solid food was allowed after 7:00 p.m., and no fluids after 10:00 p.m. Total 24-hour urine collections were made on each man for every day of the experimental period.

In order to insure basal conditions for metabolic tests the men retired at 11:00 p.m. in their special barracks which were outfitted with the equipment required to conduct these tests. The men were awakened at 7:00 a.m., permitted to void, and then were maintained in a basal state during the next three hours. The urine collected from 7:00 to 10:00 a.m. was used to determine the uric acid-creatinine ratio. In addition, leucocyte counts, differential counts and chamber eosinophil counts were determined on the men daily in the basal state. Basal metabolism was measured at intervals

methods are developed for assay of the "sugar" hormones (glycogenic corticoids) of the adrenal cortex, the information regarding them will be equivocal in stress studies.

The recent developments in the purification of the anterior pituitary adrenocorticotrophic hormone (ACTH) have led to its use in assessing adrenal cortical function. The effects in human beings (25, 26, 27) of ACTH are summarized in Figure 1. It has long been known that the number of circulating eosinophils falls in severe infections and other stress situations (28, 29). In addition, the changes in eosinophils produced by ACTH can be reproduced by injection of Kendall's Compound F (a so-called "sugar" hormone) (26).

The administration of ACTH has been suggested as a means of assessing adrenal cortical reserve (26). A single dose of 25 mg. of ACTH injected intramuscularly produces a fall in circulating eosinophils and a rise in the urinary uric acid-creatinine ratio exceeding 50 per cent in people with normal adrenal cortical reserve. Mild adrenal insufficiency is said to be indicated by a normal fall in eosinophils with only a small rise in the uric acid-creatinine ratio (20 to 50 per cent), whereas individuals with marked adrenal cortical insufficiency show a decrease in eosinophils of less than 20 per cent and an increase in the uric acid-creatinine ratio not exceeding 50 per cent.

Early experiments showed that removal of the thyroid gland interfered with regulation of body temperature (30, 31). Administration of thyroxin to thyroidectomized rats allowed them to survive longer in the cold than untreated thyroidectomized rats (30). It is of interest that iodine storage occurs in the thyroid gland in animals during the summer and that the gland is depleted of iodine in the winter (32). Furthermore low temperatures will produce hyperthyroidism in rats (33). While there are many studies showing a fall in basal metabolic rate on moving to hot climates, (34) there are only a few showing a rise on moving to cold climates (35). The basal metabolic rate of Eskimos is said to be 18 per cent above normal standards (36). An indirect test for increased thyroid activity by measuring the amount of thyroxin necessary to maintain normal gland weight during thiouracil administration showed that more thyroid hormone is secreted in cold than in heat (37).

While the thyroid gland apparently reacts to change in environmental temperature, there is considerable evidence that it plays little part in the initial defense against cold exposure. In the initial days of cold exposure in man, there is a marked fall in basal metabolism followed after the second and third day, by steady rise above control levels (38). In the rat there is evidence of a similar rise in basal metabolic rate (39, 40) but after a longer delay. The thyroid gland of the rat hypertrophies after three weeks' ex-

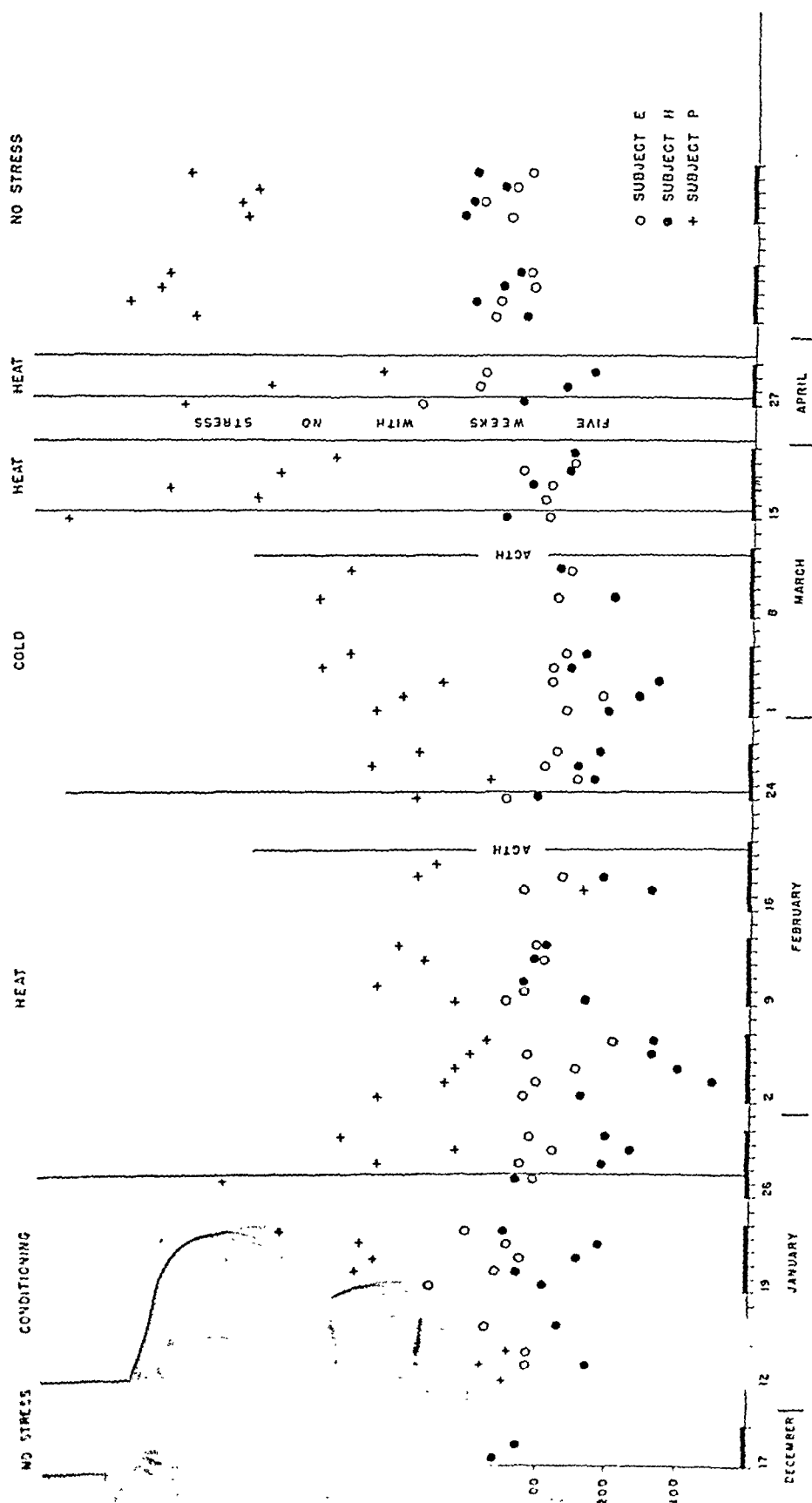


Fig. 2. Counts of circulating eosinophilic leucocytes during heat and cold stress.

of several days. On the intervening days, blood volume and "thiocyanate space" were determined.

Experimental Conditions in the Climatic Chambers

Physical conditioning was accomplished by walking the men for two hours on a treadmill at 3.5 m.p.h. for ten days, in a constant temperature room maintained at 68°F. with a relative humidity of 40 to 50 per cent and a wind velocity of 3 m.p.h. During this exercise period the men wore light clothing.

The exposures in the hot room were carried out at 107°F., dry bulb (89°F., wet bulb) with a wind velocity of 3 m.p.h. Each exposure lasted for five and one-quarter hours during which time each man walked on the treadmill at 3.5 m.p.h. for a period of sixty minutes and another of thirty minutes. During the remainder of the time in the chamber, the men sat quietly.

The cold room exposures lasted five hours, during which the men sat in a climatic chamber maintained at -20°F. with a wind velocity of 3 to 4 m.p.h. Each man wore skin and rectal thermocouples, as well as a heavy Arctic uniform. The men walked on the treadmill at 3 m.p.h. whenever the toe or knee temperature fell to 45°F. The duration of walking was for one or more periods of ten minutes until the toe or knee temperature had risen to 50°F. or more. This procedure eliminated the danger of frost-bite and at the same time permitted maintenance of the maximal cold stress possible under these experimental conditions.

Methods for Assessing Various Physiologic Changes

Basal metabolic studies were carried out by collecting expired air into 100 liter Tissot spirometers. Duplicate samples were collected and analyzed for oxygen and carbon dioxide by the method of Haldane (42). The uric acid was determined by Archibald's (43) modification of the method of Kern and Stransky (44) and creatinine by the method of Folin as modified by Lambert (45). The 24-hour excretion of the 17-ketosteroids was determined by a modification of the method of Callow *et al.* (46) employing the Zimmermann color reaction and the Wilson and Carter technique for the stabilization of the alcoholic potassium hydroxide (47). A semi-micromethod was established, using diethyl ether extraction in place of the more laborious carbon tetrachloride technique. This method permits the analysis of samples of 50 cubic centimeters or less.

The eosinophil counts were made by the chamber technique, using an eosin-acetone solution and a Fuchs-Rosenthal chamber (28). The absolute lymphocyte count was calculated from the percentage of lymphocytes on a coverslip smear (counting 300 to 400 cells), and the total leucocyte count was determined in the standard fashion.

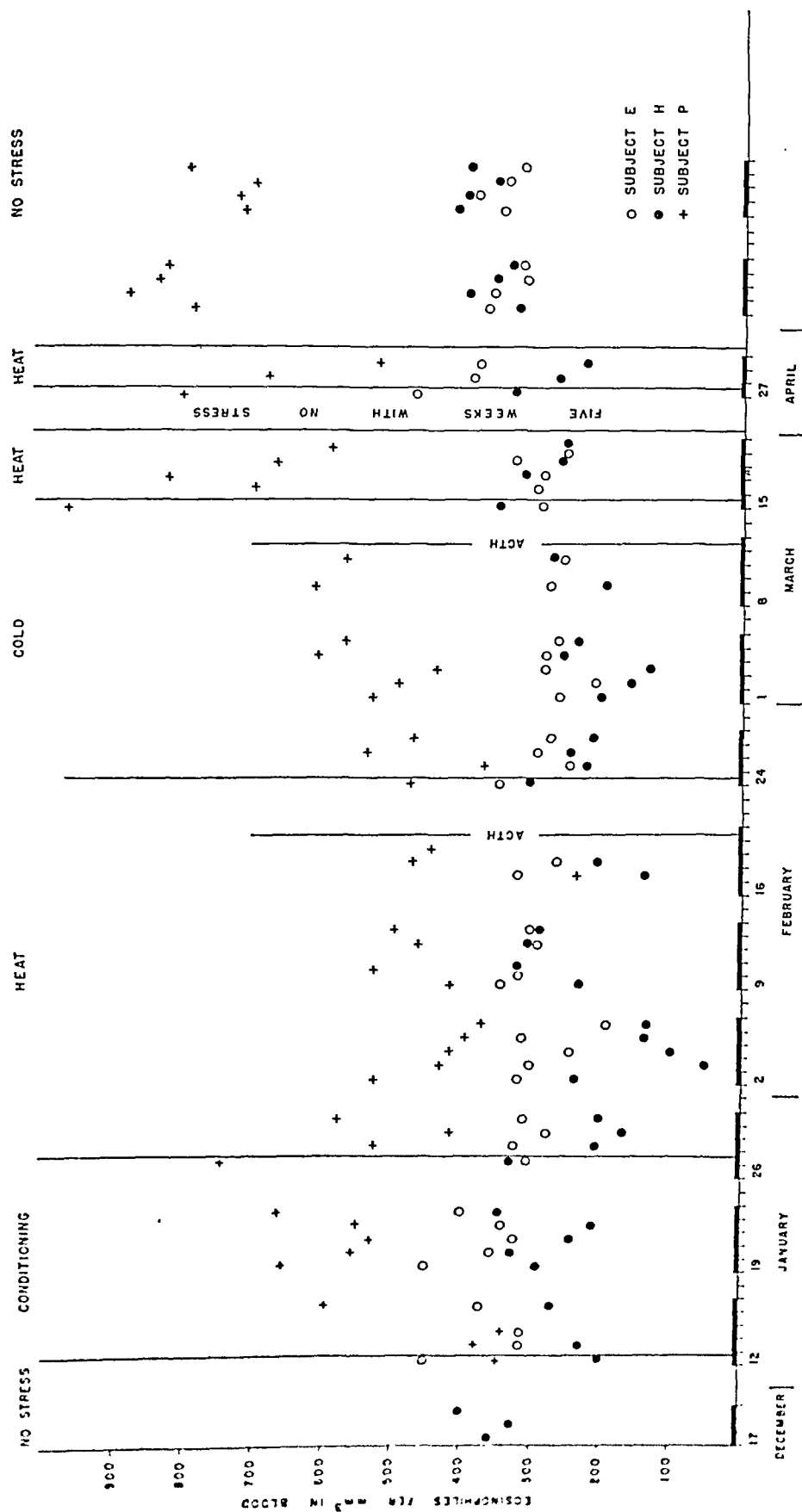


Fig. 2. Counts of circulating eosinophilic leucocytes during heat and cold stress.

TABLE 1. AVERAGE NUMBER OF CIRCULATING EOSINOPHILS PER CUBIC MILLIMETER DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Circulating eosinophils		
		Average	Standard deviation	Number of observations
E	No exercise or exposure	338	26	8
	Conditioning period	363	55	10
	First heat exposure	291	38	16
	Cold exposure	274	33	12
	First re-exposure to heat	286	25	5
	Second re-exposure to heat	407	41	3
H	No exercise or exposure	363	30	8
	Conditioning period	265	54	8
	First heat exposure	214	72	15
	Cold exposure	225	49	12
	First re-exposure to heat	291	45	4
	Second re-exposure to heat	269	41	3
P	No exercise or exposure	769	59	8
	Conditioning period	512	126	9
	First heat exposure	470	109	17
	Cold exposure	511	74	11
	First re-exposure to heat	709	99	5
	Second re-exposure to heat	660	115	3

The anterior pituitary adrenocorticotrophic hormone test for the assessment of adrenal cortical reserve was conducted as outlined by Forsham and co-workers (26). The test was conducted under basal conditions and consisted of a two-hour base-line period and a four-hour test period. During the base-line period a two-hour urine sample was collected, as well as blood samples for hematologic studies, blood uric acid (43) and blood glucose (48). Then 30 milligrams of ACTH* were injected intramuscularly. One hour after injection, the test subject voided and this urine sample was discarded, but all urine passed during the subsequent three hours was collected and analyzed for uric acid and creatinine. Four hours after the injection, the hematologic studies, blood uric acid and blood glucose

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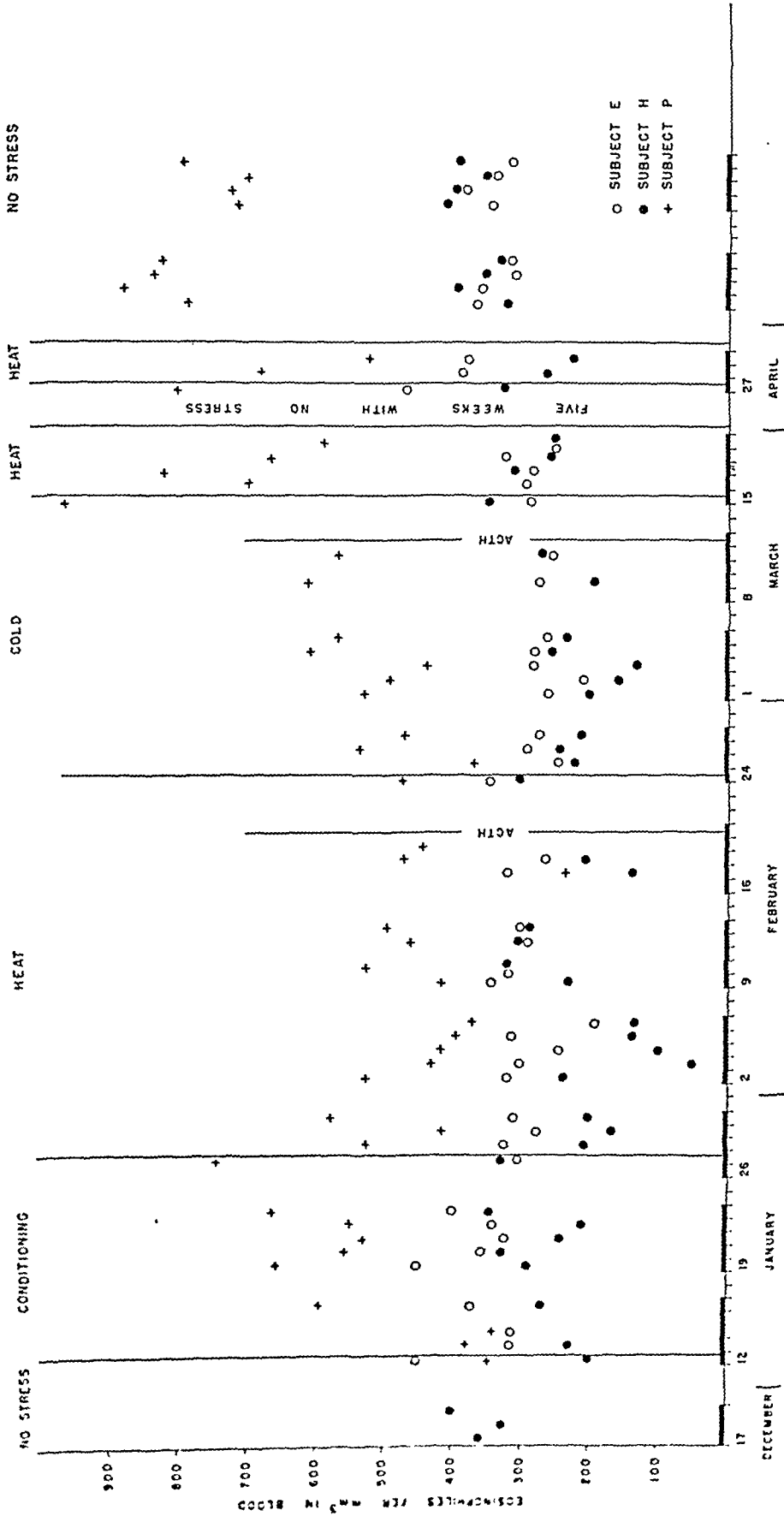


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determinations were repeated. The urine collection continued for a full twenty-four hours following injection, for the determination of the 17-ketosteroids. This test was carried out at the conclusion of the series of heat exposures, after the cold exposures, and after a period of no exposure to environmental or exercise stress. Injection of the acid solvent containing no ACTH was administered as an additional control. A 72-hour interval was judged to be sufficient to eliminate any after-effects on subsequent data, since it has been shown that the effects of a single injection of ACTH disappear within twenty-four hours (26).

EXPERIMENTAL RESULTS

It has been reported (41) that these subjects fulfilled the criteria of acclimatization to heat (49) with only slight deacclimatization to the heat after a series of cold exposures. In addition, they showed a tendency toward more rapid vasoconstriction with repeated exposures to the cold.

The results of the daily eosinophil counts are depicted graphically in Figure 2 and the average counts for each man for the various periods are shown in Table 1.

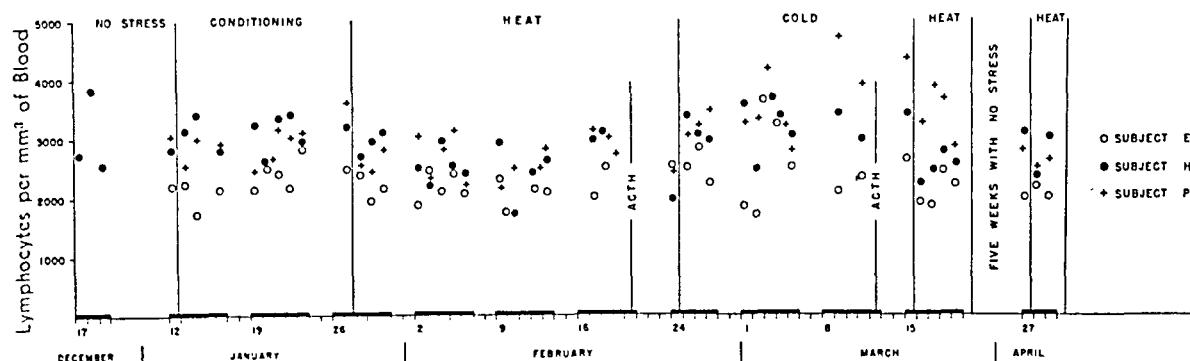


FIG. 3. Counts of circulating lymphocytes during heat and cold stress.

In addition, two of the subjects, H and P, were studied on eight days during which they were subjected to no environmental stress or exercise. These latter values were significantly higher in subjects H and P than those obtained during the initial base-line period (physical conditioning period), which is indicative of the effect of exercise on the circulating eosinophils. Likewise, in general, the number of circulating eosinophils was considerably lower during the various periods of environmental stress, particularly during the initial heat period and the cold period. In subjects H and P the eosinophil values were higher during both periods of re-exposure to heat than during the conditioning period, but lower than the values for the period of no exercise or environmental stress. The other test subject E, had a higher value during the second re-exposure than during

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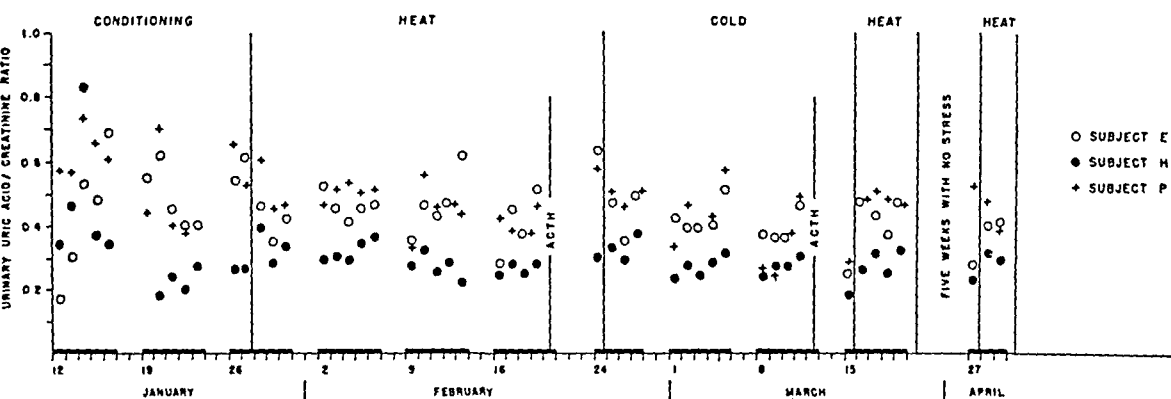


FIG. 4. Urinary uric acid-creatinine ratio during heat and cold stress.

TABLE 3. AVERAGE URINARY URIC ACID-CREATININE RATIOS DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Uric acid-creatinine ratio		
		Average	Standard deviation	Number of observations
E	Conditioning period	0.46	0.15	10
	First heat exposure	0.45	0.08	19
	Cold exposure	0.43	0.08	14
	First re-exposure to heat	0.40	0.09	5
	Second re-exposure to heat	0.36	0.07	3
H	Conditioning period	0.31	0.08	15
	First heat exposure	0.29	0.04	19
	Cold exposure	0.28	0.04	14
	First re-exposure to heat	0.26	0.06	5
	Second re-exposure to heat	0.28	0.04	3
P	Conditioning period	0.52	0.15	10
	First heat exposure	0.47	0.07	19
	Cold exposure	0.42	0.10	14
	First re-exposure to heat	0.44	0.09	5
	Second re-exposure to heat	0.46	0.07	3

The 24-hour excretion of the 17-ketosteroids was determined daily during a period of no exercise or environmental stress, during the last week of the initial heat exposure, during the cold exposure and during the two periods of re-exposure to heat. These data are shown graphically in Figure 5 and summarized in Table 4.

In the period with no exercise or environmental stress, the values are as high as or higher than those observed during the various exposure periods. There was noticeable elevation in the excretion of 17-ketosteroids dur-

the conditioning period. Under these conditions, the data suggest strongly that circulating eosinophils can be depressed by exercise or a combination of exercise and environmental stress. The degree of depression is presumably related to the severity of the stress imposed.

Simultaneously with the eosinophil determinations, absolute lymphocyte counts were made. Although other workers have reported alterations in absolute lymphocyte counts under stress conditions, no significant differences between experimental periods were observed in any of the test subjects with respect to these values (Fig. 3, Table 2).

TABLE 2. AVERAGE NUMBER OF LYMPHOCYTES PER CUBIC MILLIMETER (ABSOLUTE COUNT) DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Lymphocytes		
		Average	Standard deviation	Number of observations
E	Conditioning period	2,272	299	8
	First heat exposure	2,280	397	15
	Cold exposure	2,508	557	12
	First re-exposure to heat	2,210	345	5
	Second re-exposure to heat	2,055	85	3
H	Conditioning period	3,078	299	9
	First heat exposure	2,703	406	16
	Cold exposure	3,117	506	12
	First re-exposure to heat	2,670	453	5
	Second re-exposure to heat	2,805	341	3
P	Conditioning period	2,872	252	9
	First heat exposure	2,692	407	17
	Cold exposure	3,504	705	12
	First re-exposure to heat	3,620	564	5
	Second re-exposure to heat	2,642	150	3

It will be noted (Fig. 4, Table 3) that there was no significant difference in the daily urinary uric acid-creatinine ratio in any of the test subjects during any of the experimental periods. In connection with these studies, a group of 11 normal males were studied under basal conditions and the average value of 65 determinations on this group was 0.42 with an S. D. of 0.11. This value compares favorably with similar data reported by Forsham *et al.* (26). Analysis of each individual's own mean and variance revealed a range of means from 0.35 to 0.54, and a standard deviation peculiar to the individual.

showed a normal response as shown by the 67.7 per cent fall in eosinophils. The eosinophil fall in subject E was abnormal (29.9 per cent), whereas the other subject's fall of 45.5 per cent in circulating eosinophils is borderline and may represent a low normal response. It is interesting to note that subject E, who showed the least fall in eosinophils, had the greatest in-

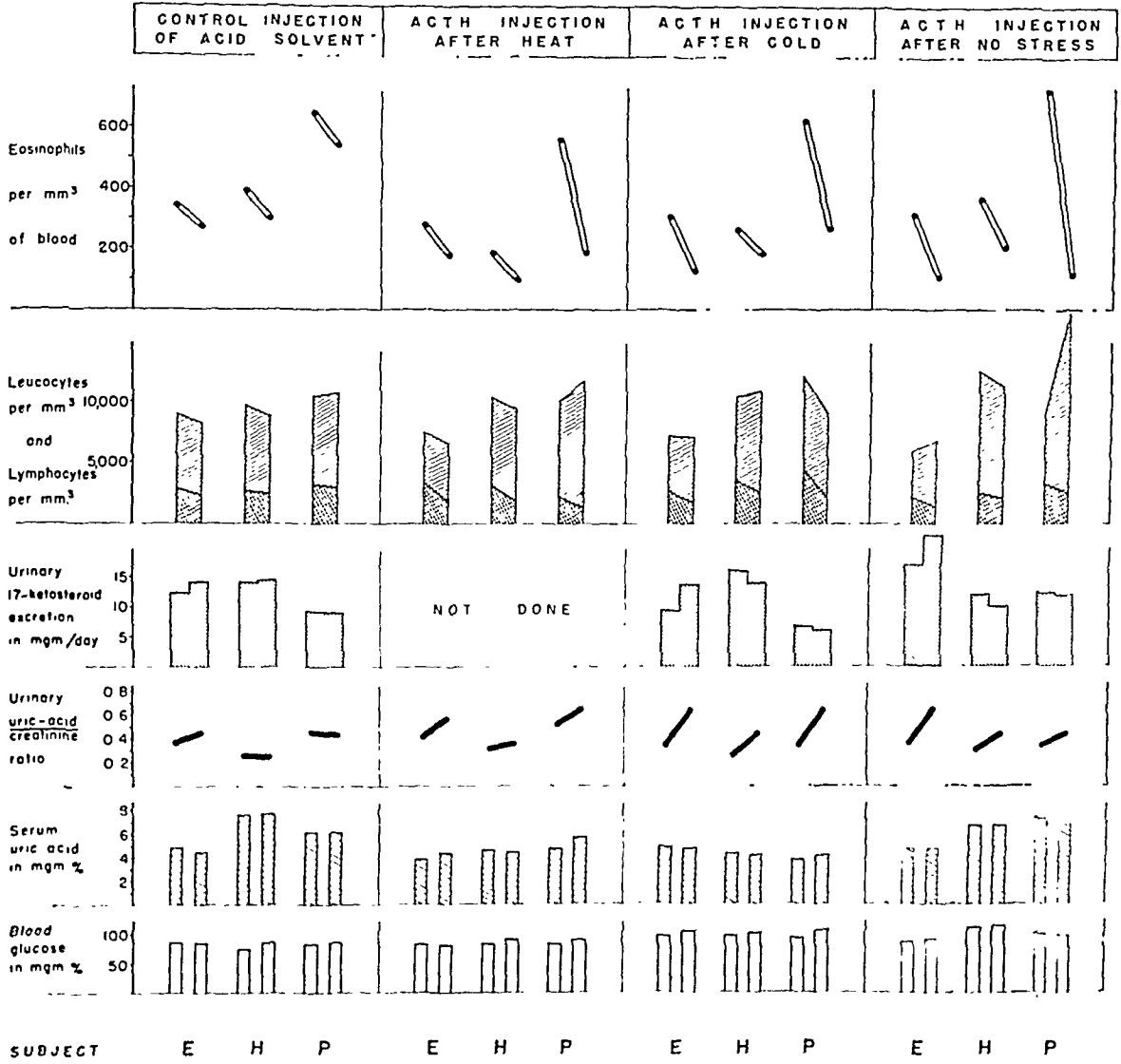


FIG. 6. Results of administration of 30 mg. of anterior pituitary adrenocorticotrophic hormone (ACTH) following heat and cold stress.

The first of each pair of results recorded for each test is that found just before injection of ACTH; the second value is that found four hours after injection.

crease in the urinary uric acid-creatinine ratio (41.5 per cent). The other two subjects showed increases of only 13.3 per cent and 15.6 per cent. Following the series of cold exposures, 2 of the 3 test subjects (E and P) had a normal fall in circulating eosinophils with increases in the urinary uric acid-creatinine ratio of 85.7 per cent and 82.9 per cent respectively. In

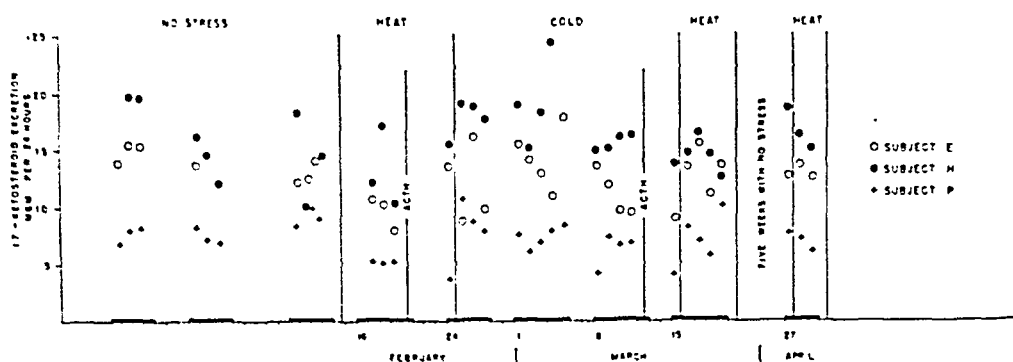


FIG. 5. Urinary 17-ketosteroid excretion during heat and cold stress.

TABLE 4. AVERAGE URINARY EXCRETION OF 17-KETOSTEROIDS (MG. PER 24 HOURS) DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Excretion of 17-ketosteroids/24 hrs.		
		Average	Standard deviation	Number of observations
E	Base-line (no exercise)	13.7	1.2	6
	Acclimatized to heat	9.9	1.3	4
	Cold exposure	12.7	2.8	14
	First re-exposure to heat	12.6	2.6	5
	Second re-exposure to heat	13.0	0.6	3
H	Base-line (no exercise)	16.4	2.9	5
	Acclimatized to heat	12.2	3.5	4
	Cold exposure	17.2	2.7	14
	First re-exposure to heat	14.4	1.5	5
	Second re-exposure to heat	16.6	1.8	3
P	Base-line (no exercise)	7.5	0.9	6
	Acclimatized to heat	5.2	0.1	4
	Cold exposure	7.1	1.8	13
	First re-exposure to heat	7.0	2.4	5
	Second re-exposure to heat	7.0	0.8	3

ing the cold exposure, as well as during the periods of re-exposure to heat when compared to the values during the last week of the initial heat exposure. These differences, however, are not statistically significant.

Results of the administration of ACTH at the conclusion of the initial period of heat exposure, at the end of the cold exposures, and after the period of no environmental stress, together with control data, are summarized in Figure 6 and Table 5. Following the heat exposures, subject P

subject H, the fall in eosinophils was only 31.6 per cent with a uric acid-creatinine ratio increase of 73.1 per cent. After a period of no exercise or exposure to extreme temperatures, subjects E and P again showed a normal decrease in circulating eosinophils (66 per cent and 86.6 per cent respectively), whereas subject H had a borderline normal response of 44.6 per cent. The increases in urinary uric acid-creatinine ratio were normal for subjects E and H (78.8 per cent and 41.9 per cent respectively) whereas subject P showed an increase of only 25.7 per cent. Injection of the acid diluting medium, with no ACTH, gave negative responses for the various indices. These appraisals of the results are based on the "normal" values published for this test by Forsham *et al.* (26). From this investigation it is not possible to conclude that "borderline" normal responses encountered after stress in these test subjects are truly indicative of a decrease in adrenal cortical reserve.

The basal metabolism of the 3 subjects throughout the various experimental periods is shown graphically in Figure 7. No significant differences were noted in any of the test subjects during the various experimental periods.

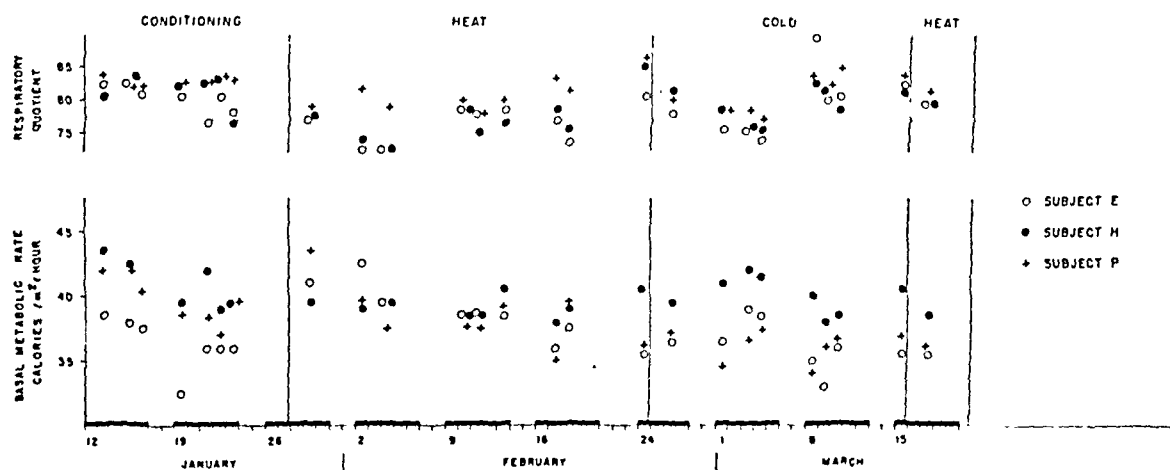


FIG. 7. Basal metabolism during heat and cold stress.

DISCUSSION

This investigation was concerned with the long-term effects of heat and cold stress. It is for this reason that stress was assessed by measurements made on the mornings following the exposures or on a twenty-four hour basis, rather than by short-term measurements of the acute effects of exposure.

Daily chamber counts of the circulating eosinophils yielded more significant data than any of the other aforementioned tests, which furnished either equivocal or negative results. The persistent decrease in eosinophils observed during these various conditions of stress, both environmental and

TABLE 5. RESULTS OF INTRAMUSCULAR ADMINISTRATION OF 30 MG. OF ANTERIOR PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH) TO THREE SUBJECTS FOLLOWING VARIOUS TYPES OF ENVIRONMENTAL STRESS

Experimental conditions	Test subject	Test condition	Leucocyte count per cu. mm.	Circulating eosinophils per cu. mm.	Lymphocyte count per cu. mm.	Urinary uric acid: creatinine ratio	Blood uric acid, mg. %	Blood glucose, mg. %	17-ketosteroid excretion, mg./24 hrs.
30 mg. ACTH after 18 heat exposures	I	Pre-ACTH	7,550	257	3,400	0.41	3.8	82.0	not done
		Post-ACTH	6,600	180	1,900	0.58	4.3	81.0	not done
		Per cent change	-12.6%	-29.9%	-44.1%	+41.5%	+13.2%	- 1.2%	
	H	Pre-ACTH	10,400	189	3,100	0.32	4.6	83.0	not done
		Post-ACTH	9,450	103	1,900	0.37	4.4	91.0	not done
		Per cent change	- 9.1%	-45.5%	-38.7%	+15.6%	- 4.3%	+ 9.6%	
30 mg. ACTH after 15 cold exposures	P	Pre-ACTH	10,100	570	2,200	0.52	4.7	82.0	not done
		Post-ACTH	11,850	186	1,300	0.65	5.7	89.0	not done
		Per cent change	+17.3%	-67.4%	-40.9%	+13.3%	+21.3%	+ 8.5%	
	E	Pre-ACTH	7,250	311	2,700	0.35	5.0	98.0	9.45
		Post-ACTH	7,200	133	1,750	0.65	4.8	106.0	13.90
		Per cent change	0	-57.2%	-35.2%	+85.7%	- 4.0%	+ 8.2%	+47.1%
30 mg. ACTH after four weeks of no exercise or extreme exposures	H	Pre-ACTH	10,500	267	3,700	0.26	4.3	98.0	16.10
		Post-ACTH	11,050	184	2,550	0.45	4.2	101.0	14.00
		Per cent change	+ 6.2%	-31.1%	-31.1%	+73.1%	- 2.3%	+ 3.1%	-9.3%
	P	Pre-ACTH	12,250	623	4,650	0.35	3.8	92.0	6.80
		Post-ACTH	9,000	270	2,150	0.64	4.2	108.0	6.05
		Per cent change	-26.5%	-56.7%	-53.8%	+82.9%	+10.5%	+17.4%	-11.0%
Control acid injection—after no stress	E	Pre-ACTH	6,000	312	2,220	0.36	4.7	87.0	17.00
		Post-ACTH	6,900	106	1,370	0.64	4.7	90.0	22.10
		Per cent change	+15.0%	-66.0%	-38.3%	+77.8%	0.0%	+ 2.7%	+30.0%
	H	Pre-ACTH	12,650	372	2,530	0.31	6.7	110.0	12.10
		Post-ACTH	11,400	206	2,165	0.44	6.7	112.0	10.00
		Per cent change	- 9.9%	-44.6%	-14.4%	+41.9%	0.0	+ 1.8%	-15.7%
Control acid injection—after no stress	P	Pre-ACTH	9,500	782	3,325	0.35	7.2	99.0	12.10
		Post-ACTH	18,400	105	2,570	0.44	6.6	96.0	11.80
		Per cent change	+94.0%	-86.6%	-22.4%	+25.7%	- 8.3%	- 3.3%	-2.5%
	E	Pre-Test	9,000	341	2,870	0.37	4.0	86.0	12.3
		Post-Test	8,250	272	2,310	0.44	4.4	85.0	14.2
		Per cent change	- 8.3%	-20.2%	-19.5%	+18.9%	- 8.3%	- 1.2%	+15.5%
Control acid injection—after no stress	H	Pre-Test	9,700	388	2,620	0.26	7.6	74.0	14.1
		Post-Test	8,800	300	2,460	0.24	7.7	86.0	14.4
		Per cent change	- 9.3%	-22.7%	- 6.1%	- 7.7%	+ 1.3%	+16.2%	+2.1%
	P	Pre-Test	10,500	654	3,250	0.46	6.2	83.0	9.2
		Post-Test	10,750	544	2,900	0.43	6.2	85.0	9.0
		Per cent change	+ 2.4%	-16.8%	-10.8%	- 6.5%	0.0%	+ 2.4%	-2.2%

that, under the experimental conditions employed, the degree or duration of cold stress might have been insufficient to produce a significantly elevated basal metabolic rate during the period of cold exposure.

SUMMARY AND CONCLUSIONS

Three healthy males were exposed successively after a preliminary two-week period of physical conditioning to 19 five and one-quarter hour periods of heat (107°F. dry bulb, 89°F. wet bulb); to 14 five-hour periods of cold (−20°F.); to 5 re-exposures to heat; to a five-week interval of no exposure to environmental stress or rigorous exercise; and finally to 3 re-exposures to heat.

Measurements of circulating eosinophils, absolute number of lymphocytes, urinary uric acid-creatinine ratio, 24-hour 17-ketosteroid excretion, and administration of ACTH were used to evaluate adrenal cortical responses. Basal metabolic rates were used as an index of thyroid activity.

Evidence indicative of a decrease in circulating eosinophils during environmental and exercise stress is presented and the relation of this observation to the secretion of the carbohydrate hormone of the adrenal cortex is discussed.

No significant differences in the excretion of the 17-ketosteroids were noted in any of the experimental periods.

Adrenal cortical function with respect to environmental stress was not successfully assessed on the basis of daily urinary uric acid-creatinine ratios or absolute lymphocyte counts, due to marked daily variations of these indices.

The use of adrenocorticotrophic hormone for the assessment of adrenal cortical reserve yielded only suggestive evidence of a reduction of adrenal cortical functional reserve after periods of environmental stress. The difficulty of interpreting these observations is discussed.

No significant differences in basal metabolic rates were observed in any of the exposure periods.

Acknowledgment

The authors are indebted to Dr. Harwood S. Belding for his advice in conducting these studies. Valuable technical assistance was furnished by Harold E. Hanson, T/Sgt. C. E. Wilson, T/Sgt. J. B. Duffy, S/Sgt. S. L. Wendkos and Corp. J. R. Jamieson. The statistical analyses were made by Miss A. M. Galligan, assisted by Corp. C. E. Hoegan. The figures were drawn by S/Sgt. A. M. Hilgendorf.

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exercise, possibly offers indirect evidence of "S" hormone activity since it has been previously reported (26) that compound F (an "S" hormone) decreased circulating eosinophils. This indirect technique may offer a new method of assessing response to chronic stress phenomena.

The excretion of 17-ketosteroids during the various stress states is slightly but not significantly elevated over control values. The observed increases fall within the range of daily variations. These data tend to confirm the observations of those workers (24) who feel that the 17-ketosteroids are not concerned with acute stress phenomena.

The urinary uric acid-creatinine ratio did not represent a good index in these long-term studies of stress because of the large daily variation. The change produced by ACTH suggests that the usefulness of the uric acid-creatinine ratio may be confined to acute stress reactions.

Studies of adrenal cortical reserve utilizing ACTH have been suggestive, but not conclusive of a decreased reserve in individuals chronically exposed to severe environmental stress. Because of the very limited number of experiments reported in this field, precise interpretation of the results is difficult. It is conceivable that the stress, although sufficient to produce adequate acclimatization to heat, was not sufficient to tax the adrenal cortical reserve. On the other hand the failure to obtain consistent results might be due to the inability of the adrenal cortex to respond to this stimulating dose, and larger, or repeated, doses would be required to produce more consistently maximal changes in normal subjects. In the present studies considerable variation was encountered in two most sensitive indices, *i.e.*, circulating eosinophils and urinary uric acid-creatinine ratio. A "normal" change in the former was often accompanied by a minimal change in the latter and vice versa, thus indicating some of the difficulty in the interpretation of results.

It has been reported that there is a decrease in basal metabolic rate in the initial phases of heat acclimatization (38, 50) but the results of the present study fail to support these observations. It is believed that the difference in results is not explained on the basis of the degree of heat stress imposed, because in the present investigation, adequate acclimatization by the accepted standards was obtained in seven to ten exposures. It is possible that the increment of change observed previously is too small to be observed consistently, and therefore these changes were not seen in the men used in this study. There are variations both in the heat and cold periods on a day-to-day basis for all of the test subjects, but there is no consistent trend. Likewise, in the cold, no significant changes were observed and this confirms previous investigations (51), although there are reports of increased basal metabolism in human beings exposed to cooler environments (35), as well as in animals subjected to cold stress. It must be borne in mind

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CHANGES IN URINARY URIC ACID-CREATININE RATIO AFTER ELECTRICALLY INDUCED CONVULSIONS IN MAN

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RECENT studies have provided evidence indicating that increased secretion of adrenal cortical steroid hormones occurs in patients given electroconvulsive therapy for mental disease (1-7). Changes in the uric acid-creatinine ratio four hours after the injection of adrenocorticotrophic hormone (ACTH) has been suggested as a measure of adrenal cortical function (8) (9) and accordingly it was considered desirable to measure this ratio in patients given electroshock therapy.

MATERIAL AND METHODS

Fifteen studies were made on 9 women who were given electroconvulsive therapy for mental disease. The ages ranged between 25 and 73 years and the diagnoses varied (Table 1).

The patients voided an hour and a half before the treatment; this urine sample was discarded and another sample was obtained immediately before shock was administered. Thereafter, samples were collected four hours after the treatment and whenever possible at the end of two hours also. In addition, some of the patients voided at other times during the four hours after shock was given; some of these samples of urine were obtained and the times at which they were voided were recorded. In other instances, however, specimens were lost, since the patients, psychotic to begin with and confused after the treatment, voided without attracting the attention of a nurse. In 7 of the 15 experiments all samples of urine voided during the first four hours after shock were collected at intervals accurately recorded; in these 7 instances calculation of hourly excretion of uric acid and of creatinine was possible. In all 15 studies the uric acid-creatinine ratio was calculated from measurements of the concentrations of these two substances in the samples of urine obtained.

Creatinine was estimated by means of Folin's method (10); uric acid was measured according to the method of Benedict and Franke, modified by Folin (11), after the procedure was adapted to the Coleman spectrophotometer.

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OBSERVATIONS

Uric Acid-Creatinine Ratio: Before treatment, the ratio was between 0.29 and 0.71, and averaged 0.45 (Table 1). The standard deviation was 0.11.

One half to two hours after check, the ratio lay between .25 and .67 and averaged 0.40; the standard deviation was 0.12. In 8 instances decreases

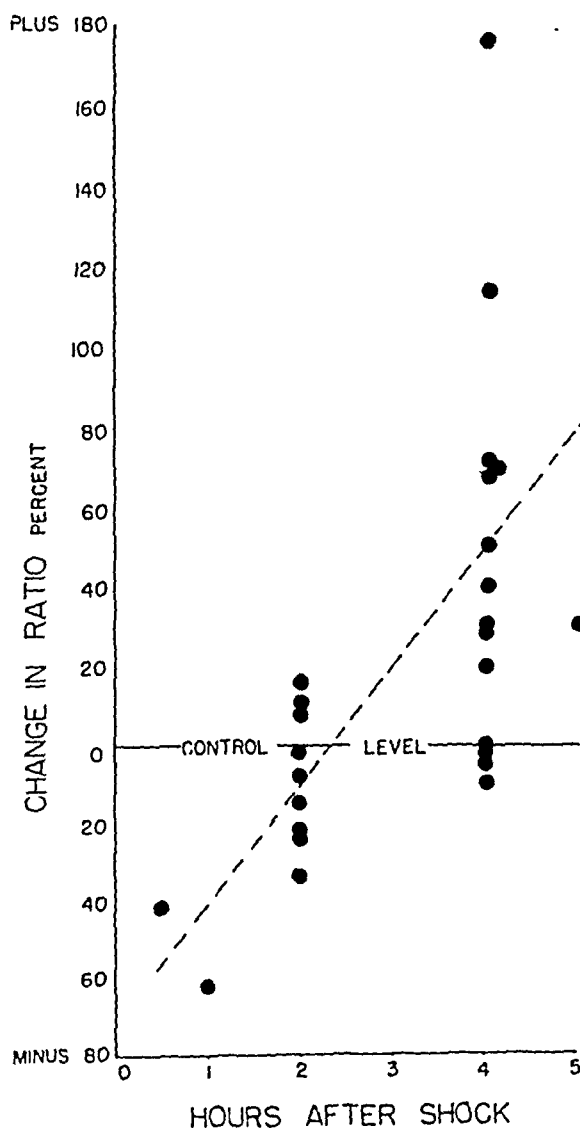


FIG. 1. Relation between change in urinary uric acid-creatinine ratio and time of study after electroshock.

of between 0.01 and 0.34, averaging 0.13, occurred. In 3 instances increases in the ratio of 0.05 to 0.11, averaging .06, were found. The average change in the 11 studies in which urine samples were obtained between one half and two hours after electrically induced seizures, was minus 0.08.

Four or five hours after administration of electroshock, the uric acid-

TABLE 1. EFFECT OF ELECTROSHOCK ON URINARY URIC ACID-CREATININE RATIO

Case	Age (yrs.)	Diagnosis	Duration of disease	Ratio	Remarks
1	50	Schizophrenia-paranoid	8 years	0.36	Before first shock.
				0.47	4 hours after first shock.
				0.29	Before second shock.
				0.50	4 hours after second shock.
2	25	Schizophrenia-paranoid	2 years	0.32	Before sixty-second shock.
				0.25	2 hours after sixty-second shock.
				0.45	4 hours after sixty-second shock.
				0.29	Before sixty-fifth shock.
3	57	Schizophrenia-paranoid	25 years	0.48	Before first shock.
				0.28	$\frac{1}{2}$ hour after first shock.
				0.63	5 hours after first shock.
				0.57	Before third shock.
4	40	Schizophrenia-paranoid	4 years	0.40	Before first shock.
				0.43	2 hours after first shock.
				0.48	4 hours after first shock.
				0.43	2 hours after third shock.
5	44	Manic-depressive, mixed	9 months	0.56	Before sixth shock.
				0.67	2 hours after sixth shock.
				1.20	4 hours after sixth shock.
				0.47	Before third shock.
6	44	Manic-depressive, manic	3 months	0.40	2 hours after third shock.
				0.71	4 hours after third shock.
				0.49	Before second shock.
				0.33	2 hours after second shock.
7	59	Involutional psychosis, melancholia	4 months	0.64	4 hours after second shock.
				0.71	Before third shock.
				0.27	1 hour after third shock.
				0.69	4 hours after third shock.
8	65	Involutional psychosis, melancholia	1 month	0.48	Before first shock.
				0.47	2 hours after first shock.
				0.81	4 hours after first shock.
				0.39	Before second shock.
9	72	Involutional psychosis, melancholia	6 months	0.36	2 hours after second shock.
				1.08	4 hours after second shock.
				0.48	Before second shock.
				0.43	4 hours after second shock.
				0.51	Before fifth shock.
				0.56	2 hours after fifth shock.
				0.51	4 hours after fifth shock.

In the urine samples collected four hours after shock the uric acid excretion per hour was between 24 and 326 per cent above the control value (Table 2). The average increase above the control values was 161 per cent. At the same time the hourly excretion of creatinine was decreased by 4 and 6 per cent in 2 instances and increased by 46 to 316 per cent in 5; the average change from the control values was an increase of 102 per cent (Table 2).

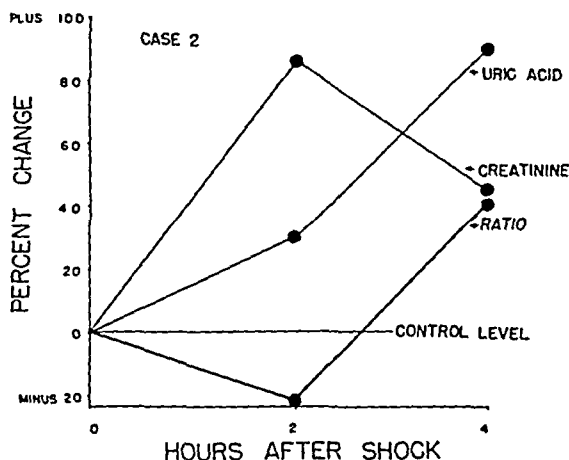


FIG. 2. Uric acid and creatinine excretions per hour after electroshock.

DISCUSSION

The studies of Thorn and of Forsham and their co-workers (8) (9) have shown that the urinary uric acid-creatinine ratio increases four hours after stimulation of the adrenal cortex by pituitary ACTH. The utilization of changes in the urinary uric acid-creatinine ratio after injection of ACTH was suggested by Thorn and his co-workers as a device whereby increased output of uric acid might be detected without the necessity for collecting every specimen of urine. When adrenocorticotrophic hormone is injected the ratio becomes elevated because the excretion of uric acid four hours later is increased greatly, while the output of creatinine remains unchanged or diminishes slightly. After electrically induced convulsions, however, the output of both uric acid and creatinine is increased markedly; the output of creatinine may show its greatest increase in the first two hours after administration of electroshock whereas as a rule that of uric acid exhibits its most marked rise in the second two hours. Since the two vary independently after electroshock, it is apparent that the interpretation of changes in the ratio is not exactly the same as in the case of changes after injection of ACTH.

Changes in ratio two hours after electroshock are not statistically significant although in most instances a decrease occurs. Four hours after the shock, however, the average rise in ratio amounts to almost twice the

creatinine ratio was between 0.43 and 1.20, and averaged 0.64; the standard deviation was 0.22. Increases in ratio of 0.08 to 0.71, averaging 0.27 occurred in 11 instances. No change occurred in 1 and decreases of 0.01 to 0.05 occurred in 3. The average change in all 15 experiments was plus 0.19 or 42 per cent of the control average value.

No clean-cut relation between time of voiding of urine after shocks and percentage change in ratio was found although the data indicate the more common occurrence of large changes when the samples were collected later in the period of study (Fig. 1).

TABLE 2. URINARY EXCRETION OF URIC ACID AND OF CREATININE AFTER ELECTROSHOCK

Case	Uric acid excretion					Creatinine excretion				
	mg. per hour			per cent change		mg. per hour			per cent change	
	Before shock	Up to 2 hours after shock	2 to 4 hours after shock	Up to 2 hours after shock	2 to 4 hours after shock	Before shock	Up to 2 hours after shock	2 to 4 hours after shock	Up to 2 hours after shock	2 to 4 hours after shock
2	10	13	19	+ 30	+ 90	28	52	41	+ 86	+ 46
5	14	43	31	+207	+122	27	64	26	+137	- 4
6	8	17	33	+112	+313	16	42	47	+163	+194
7	7	16	16	+129	+129	13	44	24	+238	+ 85
	8	9	34	+ 13	+326	12	36	50	+200	+316
8	13	17	34	+ 31	+162	35	46	32	+ 36	- 6
9	10	11	18	+ 10	+ 80	19	19	35	0	+ 84

Excretion of Uric Acid and of Creatinine: Calculation of the hourly excretions of uric acid and creatinine was possible in 7 experiments. Hourly excretions were markedly variable.

In urine samples collected at one or two hours after shock, the uric acid excretion per hour was between 13 and 207 per cent above the control value; the average was 76 per cent above the control (Table 2; Fig. 2). At the same time the hourly excretion of creatinine was between 0 and 238 per cent above the control value, the average increase being 123 per cent (Table 2; Fig. 2).

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standard deviation of the control average. In instances in which the ratio falls initially, the later rise in ratio may be so small in some cases that it does not go much above the control level four hours after shock, *i.e.*, when the effects of adrenal cortical stimulation are believed to be maximal.

The variations in ratio which occur are probably consequent, at least in part, to changes in adrenal cortical function, for other evidences of increased secretion of 11-17-oxysteroids are available; these evidences consist in decreases in the number of circulating lymphocytes (1) (6) and eosinophilic cells (5). On the other hand, it is apparent that other influences also operate to modify the urinary uric acid-creatinine ratio by increasing creatinine excretion after electrically induced convulsions. Studies of blood uric acid levels may be helpful in evaluating more precisely the significance, in regard to adrenal cortical function, of the changes in uric acid output after electroshock.

The pattern of changes found did not appear to be influenced by the patients' ages or the nature or duration of their illnesses.

SUMMARY AND CONCLUSIONS

The urinary excretion of uric acid is increased after electrically induced convulsions in man; this change is usually maximal approximately four hours after administration of electroshock and thereby resembles findings in man after the injection of ACTH. Excretion of creatinine also increases, but this is often most marked during the first two hours after shock. The urinary uric acid-creatinine ratio, therefore, remains unchanged or may fall at first, after which it rises to a level usually well above the control; in some instances, however, the late rise is not very large. The conditions of the present study are so different from those encountered by Thorn, Forsham and associates (8, 9) when they proposed that changes in the ratio four hours after injection of ACTH be used as a test of adrenal cortical function, that precise interpretation of our findings is difficult. Nevertheless, the magnitude of the changes in ratio four hours after electroshock, together with other evidence cited here, indicate increased production of 11-oxysteroids after electrically induced convulsions in man.

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tieth day of the menstrual cycle there were 20 U.R. (20 international units) of estrogens in the urine. Other clinical and laboratory data are not pertinent to this presentation.

DISCUSSION

This patient with Addison's disease, showing disappearance of axillary and decrease of pubic hair, in whom the estrogenic activity of the ovary was normal, provides additional evidence in favor of the part played by an adrenal cortical hormone in the development of these secondary sex characteristics in women. Albright and co-workers believe that it is testosterone or some similar hormone of adrenal origin. According to these authors, the favorable effect which estrogen therapy has on the growth of axillary and pubic hair in the syndrome of "ovarian agenesis" is due to a stimulating effect upon the adrenal cortex, obtained indirectly through the hypophysis. In spite of the decided influence of the adrenal cortex on the growth of the axillary and pubic hair in women, we believe that further research is necessary before we can discard the possibility of the estrogens acting directly and synergistically in combination with the cortical hormone.

With our associate Albrieux, we are at present studying the effect of applications on the skin of the axillae, of ointments containing either estrogens, testosterone, or both.

SUMMARY

This case report deals with a woman, aged 43, suffering from Addison's disease, in whom axillary hair had disappeared and pubic hair was very scanty, in spite of the fact that she menstruated and had a normal content of estrogens in her urine. The part played by the adrenal cortex and estrogens in the development of these sexual characteristics is discussed.

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LOSS OF AXILLARY AND PUBIC HAIR IN A PATIENT WITH ADDISON'S DISEASE AND REGULAR MENSTRUATION

A CASE REPORT

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ALBRIGHT and co-workers (1) in 1942 reported the case of a woman suffering from Addison's disease in whom axillary and pubic hair had not developed, in spite of the fact that she menstruated regularly from the age of 16 years. This observation led them to suppose that the sex characteristics in question are not dependent, in the woman, upon the action of estrogens but upon that of some hormone of the adrenal cortex. This belief was further supported by the following facts: It is known that the administration of estrogens does not result in the development of the aforementioned sex characteristics in patients with panhypopituitarism, in which condition there is an insufficiency of the adrenal cortex; whereas estrogens have a very favorable effect in primary ovarian insufficiency, a condition in which the adrenal cortex functions more or less normally. In thyroid insufficiency in women, scarcity of axillary and pubic hair is to be seen, attributed by Albright and associates to the hypofunctioning adrenal cortex observed in this condition, as shown by the decrease in the excretion of 17-ketosteroids. Other writers have published observations which show the importance of the adrenal factor in the development of these sex characteristics. Kepler and co-workers (2) reported a case of a woman with Addison's disease who had very scanty axillary and pubic hair, in spite of having menstruated regularly from the age of 13 years. Mussio Fournier and Proto (3) have published a similar case report.

We here give the case history of another patient of the same type.

CASE HISTORY

Mrs. V. C., a nullipara, aged 43, had a past history of little interest. Puberty was normal and she menstruated regularly with 28/3 rhythm. Her present condition began eight months previously with nausea, vomiting, weakness and darkening of the skin. Physical examination showed pigmentation of the skin and of the buccal mucosa. Her blood pressure was 90/60. There was no axillary hair and but scanty pubic hair. The urinary excretion of 17-ketosteroids was 5.4 mg. in 24 hours. On the twen-

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Urinary excretion

The demonstration of estrogens in the urine of pregnant women by Aschheim and Zondek (10) facilitated the isolation and identification of crystalline estrone, estradiol and estriol. The urine of pregnant mares and of stallions is also a rich source of the natural estrogens. Equilin, hippulin and equilenin have been obtained from the urine of the pregnant mare. However, most physiologic investigations have been confined to the human and laboratory animals. It has been demonstrated that although the estrogens are excreted in minute amounts in the normal female, there are two peaks of excretion, one at mid-interval which is considered to bear some relationship to ovulation and the other preceding the menses (D'Amour (11), Werner (12), Gustavson *et al.* (13), Gallagher *et al.* (14), Smith, Smith and Pincus (15), Salter, Humm and Oesterling (16), Jailer (17, 18) and others). There is also a fairly constant excretion of estrogens in the male (Werner (12), Gallagher *et al.* (13)). Due to differences in the technique of bio-assay employed by the various investigators, the exact quantities obtained vary. Slightly higher values have been obtained by some of the recently developed chemical methods (Salter, Humm and Oesterling (16), Jailer (18)). But generally speaking, the equivalent of approximately 5–20 micrograms of estrone as determined by bio-assay are excreted daily by the female between the peaks, at which time the excretion may rise to as high as 60–100 micrograms. The daily urinary excretion in the normal male has been found to vary between 5 and 25 micrograms.

Due to the comparative ease with which these steroids can be extracted from the urine as compared to blood and tissue, the study of urinary excretion has been used as a tool in elucidating certain aspects of estrogen metabolism. Westerfeld and Doisy (19), Fish and Dorfman (20, 21), Heard and Hoffman (22, 23), Pincus and Pearlman (24), Stroud (25), Stimmel (26), Jailer (18) and others have shown that following the injection of varying amounts of estradiol and estrone, a very small percentage of the administered dose can be recovered in the urine. A somewhat similar amount has been found in the feces (Dingemanse and Laqueur (27)). The same phenomenon occurs whether the estrogen is injected into a male or a female. The presence or absence of the end organ (uterus and tubes) or ovaries does not change the percentage of recovery. Previously, Pincus and Zahl (28) had thought the presence of the uterus was necessary for the conversion of estrone to estriol, but subsequent studies have shown this to be untrue (Pearlman and Pincus (29)). Thus, only 2–10 per cent of the administered estrogen can be accounted for and the remainder is either stored or destroyed in the organism. Storage of the administered estrogens by the organism is not an important factor (Zondek (30)), consequently

Endocrine Review

THE METABOLISM OF THE ESTROGENS: A REVIEW

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THE isolation of the estrogens by Doisy *et al.* (1) and Butenandt (2) aroused considerable interest in their metabolism (origin, action and degradation). Such information is necessary as a guide for their more rational use in therapy, as well as for more academic reasons. As far as the female genital tract is concerned, the estrogens are the most potent known stimulators of growth and cellular division. For example, by means of the colchicine technique, Allen, Smith and Gardner (3) have demonstrated as many as 1585 mitotic figures in one cross section of epithelium of the mouse vagina thirty-seven hours after the administration of but a few micrograms of estrone. It has also been shown that estrogenic stimulation, either endogenous or exogenous, is necessary for the development of certain genital tumors in strains of genetically susceptible animals (Lacassagne (4), Lipshütz (5)). There has been presented some circumstantial evidence that has implicated the estrogens as possible etiologic agents in uterine and mammary carcinoma in the human as well (Corscaden and Gusberg (6), Ayre and Bauld (7), Auchincloss and Haagensen (8)). Consequently a better understanding of the metabolism of these steroids may shed light on some of the very fundamental problems of cellular growth and division as well as neoplastic transformation.

The estrogens have been found in the ovary, placenta, urine and in some species of plants. MacCorquodale, Thayer and Doisy (9) have isolated estradiol from sow's ovary and it is postulated that α -estradiol is the steroid secreted by the ovary. The estrogenic activity of α -estradiol is about 8-10 times that of estrone and 10-15 times that of estriol when assayed by the vaginal smear method on the ovariectomized rat. With other types of assay the comparative values vary somewhat more. (In the human, α -estradiol does not appear to be that much more potent than estrone). The minimum amount of α -estradiol necessary to cause vaginal cornification in the spayed rat is modified by factors such as the strain of rat, the diet, and the housing conditions, but appears to be approximately 0.1 microgram.

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(42)). It has about 1/100 the estrogenic activity of α -estradiol. It has not been found in human urine (Heard and Hoffman (23)).

The difficulty in isolating these metabolic products is great because they occur in but minute amounts, have no estrogenic activity, and consequently cannot be detected by the usual bio-assay. Neither can they be detected by the colorimetric or the fluorometric methods. The former depends upon the presence of a phenolic "A" ring and the fluorescence upon a double bond in the "A" ring (Jailer (18)). Recoveries of urinary estrogens after the administration of exogenous estrogens are of the same order of magnitude by these methods as by bio-assay (Stimmel (26), Jailer (18)). This suggests that one method of degradation must be the destruction of the "A" ring. The solution of this complex problem may occur when it becomes possible to substitute a radioactive carbon directly into one of the rings of the cyclopentanoperhydrophenanthrene nucleus.

The role of the liver in the inactivation of estrogens

Zondek, in 1934 (30, 43), demonstrated the great facility with which the rat can inactivate estrone. It was shown that four hours after the injection of 40,000 m. u. of estrone, he could recover but 5 per cent of the administered dose in the entire animal carcass. The estrogen was practically all transformed before it showed any evidence of having exerted its biologic effect. After hydrolysis, the percentage of recovery was increased somewhat. Heller (44, 45) further demonstrated that liver slices could inactivate estrone and estradiol *in vitro*. The kidney had somewhat the same effect. The spleen, ovary, heart, lung, placenta and endometrium of the rat, on the other hand, enhanced the biologic activity of estrone and its conversion to estradiol was postulated. This exceedingly important point has not been confirmed (Twombly and Taylor (46)). The enzymatic inactivation could be inhibited by heat and sodium cyanide. Singher *et al.* (47) confirmed the *in vitro* inactivation by the rat liver while Twombly and Taylor (46) showed that the transformation mechanism in the human liver was not as efficient as in rat liver. It should be noted that these experiments were carried out under very exact and specific conditions of concentration of tissue and estrogen and there has never been demonstrated any inactivation of large amounts of steroids such as occurs *in vivo*. Liver mince is not as effective as slices in performing this inactivation of α -estradiol, suggesting that the enzyme system involved is dependent upon cellular integrity. It has recently been demonstrated (DeMeio *et al.* (48), Coppedge *et al.* (49, 50)) that nicotinamide and diphosphopyridine nucleotide (cozymase) enhance the activity of liver mince. Israel, Meranze and Johnston (51) added estrone to the perfusate of a liver, heart-lung preparation and obtained inactivation of the estrogen. However, if the liver was excluded from the

the great bulk of the estrogens is degraded or converted to substances which have no biologic activity. This mechanism is not easily saturated; for example, Heard and Hoffman (22) have injected as much as 300 mg. of estradiol into a normal male and still recovered about 10 per cent of the amount administered. It has been demonstrated also that estrone can be converted to estradiol, estradiol to estrone and both to estriol. Several investigators have not been able to isolate estriol after the administration of the other steroids mentioned (Heard and Hoffman (23)).

One of the known mechanisms of inactivation is conjugation with glucuronic acid (estriol in man) or sulfuric acid (estrone in the mare and man) (Schachter and Marrian (31), Cohen, Marrian and Odell (32)). The glucoside and ester linkages are broken by acid hydrolysis of the urine prior to extraction with organic solvents. In the urine of normal individuals the nonconjugated fraction is very small (Glass, Edmondson and Soll (33)). It has been claimed that the content of free estrogen is high in pregnant women only during the week preceding delivery (Cohen, Marrian and Watson (34)). The mechanism of conjugation does not account for the great loss of administered estrogen, however, since in most of the experiments cited above acid hydrolysis was performed on the urines.

Smith and Smith (35, 36, 37) have shown that the estrogenic activity of urine can be increased by boiling the urine with hydrochloric acid in the presence of an excess of zinc dust. They have postulated that not only is estrone converted to estradiol (which is about 10 times more active biologically) by this procedure but that some unknown degradation product is reduced back to a biologically active estrogen, "unaccounted-for T_{2n} activity." This has been confirmed by Stimmel (38) who employed a colorimetric method (Kober) for determining the estrogens. Another possible degradation product is Westerfeld's lactone (39) which has been prepared *in vitro* by the oxidation of estrone by hydrogen peroxide. Although the lactone is only 1/7 as active as estrone in stimulating the rat vaginal epithelium, it is supposedly capable of causing the release of the gonadotropic and adrenotropic factors of the pituitary (Smith (40)). Marker and associates (41) claim to have isolated estranediol from urine. It was thought to be a biologically inactive degradation product of estrone, caused by hydrogenation of the "A" ring.

Finally, Fish and Dorfman (21) have isolated β -estradiol from the urine of rabbits which had received α -estradiol by injection. (β -estradiol¹ was originally isolated from mare's urine by Hirshmann and Wintersteiner

¹ Originally, α -estradiol was considered the trans isomer and β -estradiol its cis isomer. However recent work has cast some doubt on the reality of these spatial configurations. Details of this are beyond the scope of this review.

within the body as a result of decreased inactivation of these steroids (Bean (63), Lloyd and Williams (64)) and decreased androgen production, for which there is some evidence (Glass, Edmondson, and Soll (60), Lloyd and Williams (64)). Gynecomastia was noted in American soldiers who were prisoners of the Japanese. They suffered from malnutrition and vitamin deficiencies. However, although the urinary excretion of 17-ketosteroid was low, estrogen excretion appeared normal (Klatskin, Salter and Humm (65), Salter, Klatskin and Humm (66)). Vascular spiders and palmar erythema do occur during pregnancy when the estrogen content of the blood is exceedingly high.

Cantarow, Paschkis, Rakoff and their collaborators (67, 68, 69, 70) have postulated the existence of a very interesting mechanism in the metabolism of estrogens. They have presented evidence to show the existence of an entero-hepatic circulation similar to that which exists with the bile salts. For example, they have demonstrated higher concentrations of estrogens in human bile in pregnant women at term than in the blood. Estrone has also been isolated from the bile of pregnant cows (Pearlman *et al.* (71, 71a)). Twenty-four to forty-eight hours after the injection of estradiol the bile still contains high concentrations, while none could be found in the portal or hepatic vein blood or in the urine of the dog. Absorption of estradiol from the duodenum of the dog was also demonstrated. After the implantation of a 15 mg. pellet of estradiol into the spleen of a dog, no estrogenic substance could be found in the urine but 178 micrograms could be detected in the bile on the twenty-third day. Thus, it is postulated that the estrogens are not immediately inactivated by the liver but that they are secreted into the bile, flow into the duodenum, and are reabsorbed with the bile salts into the liver. In this fashion there is an entero-hepatic circulation with a gradual degradation of these steroids. Longwell and McKee (72) could not confirm these results and showed a greater amount of estrogen in the urine than in the bile of dogs which had received 1.0 mg. of estrone. The activity in the bile was in the nonketonic fraction (estradiol) while that in the urine was in the ketonic (estrone) fraction. It is postulated that estrone is converted to estradiol in the liver, where it is then degraded.

Interrelation between vitamins and estrogens

Estrus did not occur in rats in which the ovaries had been previously transplanted to the mesentery, a site drained by the hepatic portal system (Golden and Sevringhaus (73)). This has been confirmed in the rabbit (Engel (74)). Similarly, it was demonstrated (G. R. Biskind and Mark (75, 76)) that pellets of crystalline steroids had no estrogenic or androgenic effect when implanted into the spleen of castrated rats. When the spleen was subsequently transplanted to a site drained by the systemic circula-

circulation, the estrogen could be recovered almost quantitatively. Schiller (52) also performed perfusion experiments and obtained inactivation of estrogens in the perfusate. He demonstrated the conversion of estradiol to estrone to estriol. Stilbestrol is also inactivated by the liver but to a somewhat lesser extent (53).

Oddly enough, certain plant enzymes are capable of inactivating the naturally occurring estrogens (Zondek (54), Graubard and Pincus (55)). The enzymatic activity has been shown to occur along with plant tyrosinase, laccase and phenolase, although not identical with them. This phenomenon also suggests that the destruction of the estrogens may occur in the "A" ring. This enzymatic oxidation is inhibited by cyanide and salicylaldehyde.

Investigations in the intact organism have also demonstrated that the liver is the site of the inactivation process. Talbot (56) fed immature rats carbon tetrachloride by gavage and noticed an increase in uterine weight. Since this did not occur in ovariectomized rats, he postulated that the minute amount of estrogen secreted by the immature rat ovary is usually inactivated by the liver but in the presence of hepatic damage this process fails. Schiller and Pincus (57, 58) demonstrated an increased urinary excretion of administered estrogens, as compared to controls, in rats which were partially hepatectomized. Diet-induced cirrhosis of the liver in rats results in deficiency of the degradation mechanism (Shipley and György (59)). The results obtained with deficiency diets will be discussed subsequently.

Similar results have been obtained in the human. Glass, Edmondson and Soll (60) have shown that not only do male patients with cirrhosis of the liver excrete more estrogens than do normal men but they excrete as much as 83-86 per cent of an administered dosage of estrogen. In infectious hepatitis, the urinary excretion of estrogen is high and shows a fall during convalescence (Gilder and Hoagland (61)). There appears to be a correlation between the excretion of estrogens and hepatic damage, as evidenced by the height of the cephalin flocculation test, in infectious hepatitis. In three male patients with hepatitis the urinary excretion varied from 40-50 micrograms per day (twice normal) when the flocculation test was 4 plus. As convalescence proceeded and the flocculation test reverted to normal the excretion of estrone and estradiol decreased to within normal amounts (Jailer, unpublished data). However, in pregnant women, Zondek and Black (62) could show differences in urinary estrogen excretion only when the hepatitis was extremely severe.

Gynecomastia, vascular spiders, palmar erythema, loss of chest and axillary hair and testicular atrophy have been described in patients with cirrhosis of the liver. These physical signs may be due to excessive estrogen

Drill and Pfeiffer (89) and Jailer (90), using different methods, have been able to separate the effects of vitamin B deficiency from the concomitant inanition. Evidence was presented to show that in the presence of inanition even huge amounts of the various components of the B vitamins are ineffective in reestablishing the ability of the liver to inactivate estrogens (90). However, it is possible that these divergent results are due to differences in the strain of rats used by different investigators. Some of the animals used by Biskind (Sherman rats) came into constant estrus within a few days after being placed on a vitamin B deficient diet. On the other hand, Sprague-Dawley rats (Drill and Pfeiffer) and Long-Evans rats (Jailer) required eighteen to twenty-one days of a deficiency diet before showing constant estrus smears, although in the latter study the urinary excretion of thiamine fell to practically zero within a few days after the institution of the vitamin B deficient diet.

Another factor in the synthesis or maintenance of the estrogen inactivation mechanism of the liver appears to be the protein content of the diet. György (91) and Unna *et al.* (92) have demonstrated both *in vivo* and *in vitro*, respectively, that estrogen inactivation fails when the animals are on a low protein diet. When methionine and choline were added to the diet of these rats, estrone was again inactivated.

It has been recently shown (Jailer, unpublished data) that if the percentage of protein in the diet is high, estradiol can be inactivated by the rat liver *in vivo* even in the presence of inanition or vitamin B deficiency. Other rats on isocaloric diets containing a lower percentage of protein can not inactivate the estrogen. Thus it would appear that the protein in the diet is the limiting factor in the ability of the rat liver to degrade the estrogens.

A different type of relationship appears to exist between the estrogens and folic acid. Exhaustion of the folic acid content of the body interferes with the normal biologic action of stilbestrol so that it may be possible that in some species at least, the action of stilbestrol is mediated through an enzyme system which requires the presence of folic acid. When chicks are maintained on a folic acid deficient diet and then given stilbestrol, the characteristic increase in the size of the oviduct does not occur (Hertz (93, 94), Kline and Dorfman (95)). The normal response can be attained if folic acid is added to the diet but will not occur on addition of L. casei factor (Hertz). There is also evidence that the same phenomenon may occur in the monkey (Hertz). However, another effect of the administration of stilbestrol in the chick, namely hyperlipemia, is not influenced by folic acid deficiency (Hertz). Koref and Engel (96) have claimed that incubation of estrone with folic acid causes an inactivation of the estrone; however, confirmation of this is lacking.

tion, the estrogen or androgen absorbed from the pellet produced its specific effect on the organism (Biskind and Mark (75)). Thus, when the liver gets the first opportunity to act on the estrogen it can completely inactivate it. However, there appears to be a limit to the capacity of the liver to degrade estrogens. When multiple pellets are implanted into the rat spleen, estrus does occur (Segaloff (77)). Segaloff (78, 79, 80) demonstrated that intrasplenic injections of estrone and estradiol were much less effective in producing estrus as determined by vaginal smear than subcutaneous injection. The differences in effectiveness between the two modes of administration was less marked with estriol. However, Hooker, Drill and Pfeiffer (81) found that in the monkey, pellets of estradiol implanted into the spleen were biologically active, even in the presence of a normal functioning liver. In the rabbit and guinea pig splenic implants of crystalline steroids are inactive, demonstrating hepatic inactivation (Biskind and Meyer (82), Lipschütz *et al.* (83)). Employing the technique of splenic implantation of estrone and estradiol, Biskind and Biskind (84) found that ovariectomized rats would show signs of constant estrus in about ten days when fed a diet deficient in the components of the vitamin B complex. Thus, as a result of dietary deficiency in the components of the B complex, the liver loses its ability to inactivate estrogens. (It still retains its capacity to inactivate androgens, however.) Thiamine and riboflavin are the components necessary for the mechanism of inactivation (Segaloff and Segaloff (85), Singher *et al.* (47)). Singher and his collaborators correlated the efficiency of the degradation mechanism of the liver with its concentration of thiamine and riboflavin. M. S. Biskind (86) elaborated on this mechanism and ascribed many clinical conditions of supposed hyperestrinism, *i.e.* "functional uterine bleeding," cystic mastitis, premenstrual tension, postpartum subinvolution of the uterus, diminished libido and impotence in the male, to a decreased estrogen inactivation as a result of vitamin B deficiency. These conditions are associated with other signs of vitamin B deficiency, for example, glossitis and cheilosis. Biskind further claims that under intensive therapy with the B vitamins these conditions soon disappear. However, his therapeutic claims have not been substantiated, nor do patients with vitamin B deficiency excrete greater amounts of administered estrogen than do normal individuals as one would expect if the inactivation mechanism were impaired (Zondek and Black (63)). Ayre (87) has also claimed that thiamine deficiency and the resultant hyperestrinism occur in women with carcinoma of the cervix uteri, and has postulated an etiologic basis for the carcinoma based on these findings. This has been questioned by Jailer (88) who could find no difference in thiamine saturation between women with uterine carcinoma and selected controls.

of urinary estrogens indicates a maximum rate of estradiol to estrone to estriol conversion and greatest when the total estrogens are low and the rate of conversion is low. The former condition is most pronounced when there is evidence of progesterone secretion or may be attained by the administration of progesterone. This is seen in the toxemias of late pregnancy where the high $T_{24}:T_0$ ratio can be decreased by progesterone administration. They interpret this as indicating that certain steroids can influence the catabolic mechanism of the estrogens (105). However, Heard, Bauld, and Hoffman (106) could find no difference in the urinary excretion of estrogens in the rabbit, when estradiol was administered alone or together with progesterone. But the ratio of the doses of estrogen to progesterone given by the latter workers was much different from that of Smith and Smith. The ratio of progesterone to estrogen may modify the biologic action of estrogen (Jones and Astwood (107)).

The origin of the estrogens

The steroids are apparently synthesized by the body but little information regarding this mechanism is available. Because of the cyclopentanophenanthrene nucleus common to both cholesterol and the steroids, it has been tacitly assumed that the latter substances are synthesized from cholesterol in the gland concerned. Bloch (108), by use of the isotope technique, has demonstrated the conversion of cholesterol to pregnanediol in the pregnant woman, presumably in the placenta by way of progesterone. Sayers and Sayers (109) have correlated adrenal cortical secretion with a decrease in the cholesterol and ascorbic acid content of the adrenal, under conditions of adrenotropic hormone injection and stress. A somewhat similar phenomenon may occur in the ovary, at least as a result of gonadotropic hormone administration. The injection of pregnant mare serum and chorionic gonadotropic hormone results in a decrease of ovarian "cholesterol" in the rabbit, as shown by histochemical methods (Claesson and Hillarp (110, 111)). This observation has been interpreted as evidence that the decrease in ovarian cholesterol is correlated with the secretion of estrogen. Although Levin and Jailer (112) could show a decrease in total ovarian cholesterol, as determined chemically, in the mature rat ovary soon after the injection of chorionic gonadotropic factor, equine pituitary extract and pregnant mare serum, this decrease in ovarian cholesterol did not occur after the administration of an extract of menopausal urine. The latter gonadotropin is mostly follicle stimulating in action, whereas the former three contain luteinizing properties as well. Consequently, it is entirely possible that the decrease in ovarian cholesterol is indicative of progesterone secretion and not of the production of estrogen. Since the quantity of

The possibility of an "activating mechanism"

Not only is the liver concerned with the inactivation of the estrogens but it is possible that it plays a role in some activating process as well. Roberts and Szego (97) have found that in the first twelve to eighteen hours after partial hepatectomy, the uterine response to intravenously administered estradiol is markedly reduced in the previously spayed rat. As time goes on and the liver regenerates, this response becomes essentially normal and at forty-eight to seventy-two hours there is a supernormal peak. From these results, it could be postulated that an hepatic enzyme is necessary for estrogenic activity. However, this would not explain why there is an increased activity of the estrogens when they are applied locally. The phenomenon of the local action of hormones has been recently reviewed by Speert (98). There appears to be some activation mechanism in the animal organism. Emmens (99, 100) has demonstrated that the more common estrogens (estradiol, estrone, stilbestrol, and others) are much more potent when applied locally to the vaginal epithelium than when administered parenterally. However, on the other hand, there is a group of synthetic estrogens (such as triphenylethylene and phenyl stilbestrol) which are much more effective when injected than when applied locally. He termed the latter group "proestrogens," because they must be converted by the animal body to a more biologically active substance. Segaloff (101) has demonstrated that the liver is the site of this conversion, although triphenylchloroethylene is no less effective in a partially hepatectomized rat than in controls. He (102) has recently shown methyl bisdehydrodisynolic acid (an estrogen originally prepared from estrone by Doisy, in which the "D" ring is opened) to be more active when injected intrasplenically than when given subcutaneously. Eversole, Birnie and Gaunt (103) have demonstrated that although the liver is incapable of inactivating methyl bisdehydrodisynolic acid when pellets are implanted in the spleen, there was no enhancement of its activity as a result of its passage through the liver.

Effect of other hormones on the metabolism of the estrogens

Although the metabolism of the estrogens has been amply investigated, the influence of other hormones on this metabolism has been barely touched. Pincus and Zahl (26) have shown that when progesterone is administered together with estradiol, there is a greater excretion of the estrogen in the urine. This could mean that progesterone exerts a sparing effect on the catabolism of the estrogens. Smith and Smith (35, 36, 37, 104) demonstrated that the urinary excretion of the apparently nonestrogenic material which may be rendered estrogenic by zinc-hydrochloric acid treatment differs under varying conditions ($T_m:T_o$ ratio). It is least when the partition

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estrogen necessary for biologic activity is so minute, it is easy to see why the problem of ascertaining estrogen precursors is so difficult.

Addendum

Since the completion of this manuscript, there have appeared two publications in which the authors have employed radioactive labeled steroids. Twombly, McClintock and Engelman (*Am. J. Obst. & Gynec.* 56: 260, 1948) have prepared 7,8 dibromestrone from equilin, employing radioactive bromine. Administration of this substance resulted in a localization of the radioactive bromine in the gall bladder and intestinal tract. These observations support the theory of an enterohepatic circulation. Albert, Heard, Leblond and Saffran (*J. Biol. Chem.* 177: 247, 1949) used iodinated derivatives of estradiol consisting of mono- and diiodo- α -estradiol containing I^{131} . The outstanding feature of these observations was also the accumulation of the labeled material in the gastro-intestinal tract. The next highest concentration of radioactivity was found in the mammary glands. In neither report was there any evidence of localization of the labeled material in the ovary, uterus, vagina or liver. It should be emphasized, however, that the addition of bromine or iodine to the steroids causes a loss of estrogenic activity, so that the substances administered are not true estrogens.

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The annual stipend will be £1000 (\$4,020.00). An allowance of \$600.00 is made for travel to the site of the fellowship in Great Britain.

University staff appointment, with teaching duties agreeable to the fellow, is permitted, provided it carries no additional salary and provided it is acceptable to the Committee on Growth, the American Cancer Society, and the British Empire Cancer Campaign. No other remunerative work will be permitted during the tenure of the fellowship.

Fellowship appointments are subject to the condition that once accepted, they will not be vacated or the place of work changed within the period of tenure without the consent of the Committee on Growth, the American Cancer Society, and the British Empire Cancer Campaign.

Time of Application

Application forms for British American Exchange Fellowships in Cancer Research may be procured from and submitted at any time to the *Executive Secretary of the Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Ave., Washington 25, D. C.* Favorable action by the Committee on Growth takes the form of a recommendation to the American Cancer Society, and is subject to approval by the Society. Fellowships will be made effective at the convenience of the institution and the fellow.

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Within thirty days after the termination of each year of the fellowship, the fellow is required to submit to the Committee on Growth a report of his work during that period.

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It is understood that results of work carried out by a fellow shall be available to the public through the approved scientific channels without restriction. Publications should include a statement that the investigation was carried out during tenure of a British American Exchange Fellowship in Cancer Research of the American Cancer Society and the British Empire Cancer Campaign, recommended by the Committee on Growth of the National Research Council. The Comptroller, American Cancer Society; Executive Secretary of the Committee on Growth; and the General Secretary of the British Empire Cancer Campaign should be notified immediately of acceptance of manuscripts for publication, in order that reprints may be ordered.

LETTERS TO THE EDITOR

ETHINYL ESTRADIOL

TO THE EDITOR:

IN THE recent excellent review on *Ethinyl Estradiol* by Dr. Kenneth W. Thompson, which appeared in this Journal (8: 1088-1098 (Dec.) 1948), a statement was inadvertently made which would seem to indicate that in human subjects the material was equally potent whether given by mouth or by intramuscular injection.

We have been interested in this estrogen for some time and, as mentioned in the review, (Segaloff, A.: The intrasplenic injection of some synthetic estrogens and proestrogens, *Endocrinology* 34: 335-339 (May) 1944) have reported that, in the rat at least, there is marked hepatic inactivation of ethinyl estradiol.

Because of this, studies of the comparative oral and intramuscular potency of ethinyl estradiol were undertaken in man. A more extensive analysis of the data obtained was presented at the Laurentian Hormone Conference (Segaloff, A.: Metabolism of estrogens with particular emphasis on clinical aspects of physiology and function of ovarian hormones, *Recent Advances in Hormone Research*, Volume 4. In press).

The method of Allen (Allen, W. M.: The biological activity of various estrogens, *South. M. J.* 37: 270, 1944) was employed for assessment of the estrogen. This method involves the administration of estrogens to women in the menstrual age group who have been ovariectomized but who have intact uteri. The end point for the assay is the occurrence of uterine bleeding after a standard period of administration. Using Allen's method, we have found that our 50 per cent bleeding dose for women following oral administration is 0.35 mg. per week, which agrees well with Allen's 50 per cent bleeding dose of 0.4 mg. per week. In the same patients, further studies were undertaken with the injection method, using ethinyl estradiol dissolved in sesame oil. It was found that 0.08 mg. per week was sufficient to produce withdrawal bleeding in 5 of 10 trials. This, we feel, indicates that there is approximately a fivefold difference between the potency by intramuscular injection and the oral potency of ethinyl estradiol in the human subject. This agrees well with data reported for other estrogens administered by various routes and utilizing various other end points.

This letter is not intended to detract from Dr. Thompson's article in any way but offers additional data which as yet are unavailable in the litera-

The Virilizing Syndrome in Man

Drs. Louis J. Soffer, J. Lester Gabrilove, Joseph W. Jailer, and Mildred D Jacobs, The Mount Sinai Hospital

Friday evening, September 16

VI. MECHANISMS OF HORMONE ACTION

Effects of Hyperglycemic Factors of the Pancreas and of Epinephrine on Glycogenolysis

Dr. Earl Sutherland, Washington University, St. Louis

Saturday morning, September 17

Hormone-Enzyme Relationships

Dr. Roland K. Meyer, The University of Wisconsin

Saturday morning, September 17

Attendance at this Conference is limited by the accommodation available at the hotel, but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee on Arrangements consists of R. W. Bates, E. R. Squibb & Sons; R. D. H. Heard, McGill University; A. D. Odell, Charles E. Frosst & Co.; E. C. Reifenshtein, Jr., Sloan-Kettering Institute; A. White, School of Medicine, University of California at Los Angeles; and G. Pincus, Chairman, The Worcester Foundation for Experimental Biology. Applications for attendance at the Conference should be sent to the Chairman at 222 Maple Avenue, Shrewsbury, Massachusetts. Applications to be considered by the Committee must be received by June 6, 1949.



The Laurentian Hormone Conference

THE Laurentian Hormone Conference of the A.A.A.S. will hold its 1949 meeting at the Forest Hills Hotel, Franconia, New Hampshire, September 12 through 17. The following program has been arranged:

I. OVARIAN PHYSIOLOGY AND FUNCTION

Biology of the Ovary

Dr. A. S. Parkes, National Institute for Medical Research, London, England
Monday evening, September 12

Physiology of Estrogenic Hormones

Drs. K. E. Paschkis and A. Rakoff, Jefferson Medical College
Tuesday morning, September 13

Maintenance of the Corpus Luteum and Physiologic Actions of Progesterone

Drs. James T. Bradbury, Willis E. Brown and Laman A. Gray, State University of Iowa and University of Louisville
Tuesday morning, September 13

The Vasculature of the Ovary and Ovarian Function

Dr. S. R. M. Reynolds, Carnegie Institute of Washington
Tuesday evening, September 13

II. CHEMISTRY AND PHYSIOLOGY OF THE SEX HORMONES

Studies with Estrogen Conjugates

Drs. G. A. Grant and D. Beall, Ayerst, McKenna & Harrison, Ltd.
Wednesday morning, September 14

Chemical Methods for the Estimation of Steroid Hormones and their Metabolites

Dr. Lewis Engel, Massachusetts General Hospital
Wednesday morning, September 14

III. PITUITARY PHYSIOLOGY AND FUNCTION

Pituitary Control of Steroid Secretions

Dr. Roy O. Greep, Harvard School of Dental Medicine
Thursday morning, September 15

The Present Status of Posterior Lobe Hormones

Dr. Robert L. Noble, The University of Western Ontario
Thursday morning, September 15

IV. HUMORAL MEDIATORS IN NERVOUS TRANSMISSION

Sympathetic Neurohumors

Drs. M. L. Tainter and F. P. Luduena, Sterling Winthrop Research Institute
Thursday evening, September 15

The Acetylcholine System in Neural Function

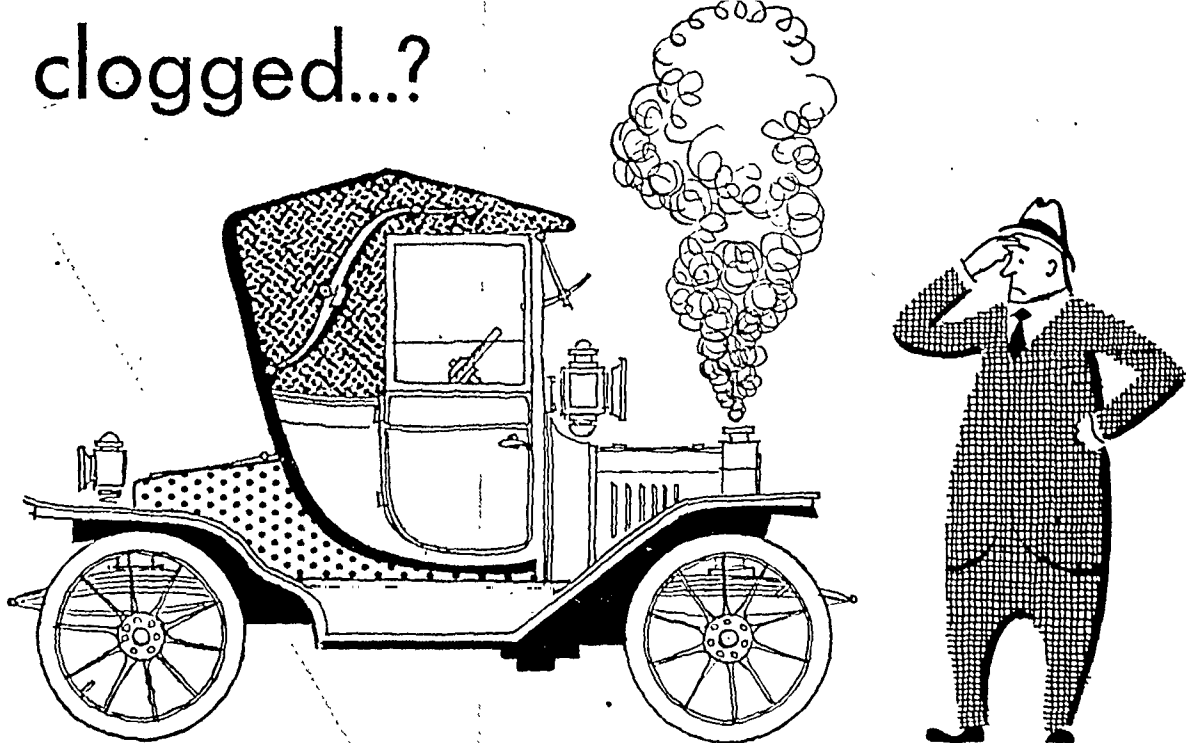
Dr. Ralph W. Gerard, The University of Chicago
Friday morning, September 16

V. HORMONES AND TUMORS

Experimental Endocrine Tumors

Dr. George W. Woolley, Roscoe B. Jackson Memorial Laboratory
Friday morning, September 16

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The Journal of CLINICAL ENDOCRINOLOGY

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of the castrate guinea pig after injection of a lipoid extract of bull testis. With suitable stains, lipids were found by him to predominate normally in the Leydig and Sertoli cells. Since the work of Fellner others have confirmed the presence of substances with estrogenic activity in testicular extracts. Brouha and Simonnet (5), Zondek (6), Dorfman, Gallagher and Koch (7) and Cunningham and his associates (8) have clearly demonstrated the presence of estrogenic substance in bull testis; Zondek, the large quantity of estrogen in stallion testis; and Laqueur and deJongh (9), the presence of estrogen in human testis. In 1940 Beall (10) isolated crystalline alpha-estradiol and estrone from horse testis.

These studies are few in comparison to the number of studies made of male urine to determine the presence or absence of estrogens. In this regard estrogens have been found in the urine of normal men, stallions, geldings and colts, as well as other male animals. In 1942 one of us with Vermeulen (11) found moderate amounts of estrogen in the urine of aging men with prostatic cancer. Generally, following castration, urinary estrogens fell to low levels; but 3 individuals showed single high postcastrational values.

That estrogen is produced by the testis seems likely from the foregoing reports of the presence of estrogen in testicular extracts and the decrease in urinary estrogens following castration. Where in the testis is estrogen produced?

With increasing time, evidence accumulates indicating that estrogen is produced by the sustentacular cells of Sertoli. It appears difficult to establish priority for this concept, but certainly Zuckerman and McKeown (12) were aware of this possibility. These authors in a study of 243 dogs found testicular tumors present in 35 of them. Fifteen of these tumors were classified as adenocarcinomata, and in 5 of these Sertoli elements predominated. These authors, aware of the concept of Witschi (13)—that the testicular sustentacular cells of Sertoli are homologous with the granulosa cells of the ovarian follicle, both arising from the follicle cells of the primordial gonad—suggested that the estrogenic potency of these tumors was related to the fact that Sertoli cells take part in their formation. To them, estrogenic potency or action was evidenced in these dogs with testis tumors by squamous metaplasia of the prostatic epithelium, enlargement of the nipples, increased pigmentation of the skin over the abdomen and stratification of the urethral epithelium, all of which occurred in control dogs injected with estrogenic substances.

In reviewing the literature Zuckerman and McKeown were impressed with the great similarity of the tumors producing feminizing changes in their dogs to the three testicular tumors in dogs reported by Greulich and Burford (14). The writers in reviewing these articles were impressed with

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ESTROGEN PRODUCTION BY THE TESTIS*†

MORGAN BERTHRONG, M.D., WILLARD E. GOODWIN,
M.D.‡ AND WILLIAM WALLACE SCOTT, M.D.

*From the Department of Pathology and the James Buchanan Brady Urological Institute,
Johns Hopkins Hospital, Baltimore, Maryland*

ONE hundred years ago Berthold (1), on the basis of transplantation experiments in the cock, presented evidence that the testes exert decided effects on the organism through the blood stream, independent of established nervous pathways. Since then tremendous energy has been expended on determining the nature of the hormones elaborated by the testis, the cells responsible for their production and the physiologic actions of the hormones produced. Primarily, workers have concerned themselves with a study of testicular substances having masculinizing effects but considerable work has been done in regard to feminizing testicular influences. Without reviewing the evidence, it seems fair to say that most workers agree that masculinizing effects are secondary to the production of an androgenic substance or substances presumed to come exclusively from the interstitial cells of Leydig independent of the generative tissue of the testis (2, 3). However, there is a considerable body of evidence to suggest that tubular activity contributes to hormone production.

Fellner in 1921 (4) appears to have been the first to suggest, on the basis of experimental findings, that the testis produced a substance with a feminizing or estrogenic action, observing an increase in uterine weight

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* Paper read at the annual meeting of the Mid-Atlantic Section, American Urological Association, Hot Springs, Virginia, March 12, 1948.

† This work was aided by a grant from the National Brewing Company.

‡ National Research Council Fellow in the Medical Sciences.

animal excreted 1.6 milligrams of 17-ketosteroids per day and 20 international units of estrogen per day. Follicle-stimulating hormone determination gave negative results in all dilutions, indicating less than 38 mouse units per day. Simply expressed: urinary estrogens were high, 17-ketosteroids low and gonadotropins not demonstrated.

Decision was made to remove the testicular tumor and to take a specimen of the prostate gland and one of the breasts for biopsy. This was begun, but unfortunately the animal died postoperatively, presumably from too much nembutal, and all tissues necessary for study were obtained immediately postmortem.



FIG. 1. Photograph of abdomen of Dog T. with the pigmented skin, prominently enlarged nipples and enlarged scrotal sac plainly demonstrated.

Autopsy

The right testis was found almost completely replaced by a tumor, $4.5 \times 4.5 \times 6$ centimeters and weighing 45 grams (Fig. 2). The epididymis was normal. Cross sections of the tumor showed hard, moist, greyish-yellow tissue in which softer, very grey tumor tissue $4 \times 3 \times 6$ centimeters, containing sanguineous fluid, surrounded the abdominal aorta. Examination of the left testis and of all other tissues save of the central nervous system, revealed nothing abnormal.

the striking similarity in histologic pattern of the adenocarcinomata reported by the above authors and the "Sertoli-cell tumor" to be described here.

In 1945 Huggins and Moulder (15) in a study of 5 dogs with feminizing testicular tumors of spontaneous origin reported the results of their investigations which included a description of the gross and microscopic anatomic status of the animal, an analysis of the tumor lipids and of the tumor estrogens. Their results clearly indicate that in the dog, feminizing tumors exist which are high in lipid content, high in estrogen content, and give the appearance of being composed predominantly of Sertoli cells which retain their normal character of accepting fat stains. They concluded that "the cells of Sertoli in the testicular tubules produce estrogen."

Recently one of us, having aided in the estrogen assays of the animals reported by Huggins, had occasion to recall his work and to institute a study of a male dog with a feminizing testicular tumor.

Case History of Dog T

This male English setter had always been well until 12 years of age. At this time it was first noticed that the hair became generally somewhat sparse and that over his lower back it was occasionally lost entirely. Local veterinarians felt that it did not represent the usual picture of "mange," but were unable to make other diagnoses. This complete loss of hair over the base of the tail occurred at intervals, with periods of regrowth, for no apparent cause and with no therapy. This area apparently was extremely itchy. About the same time, marked pigmentation of the skin of the abdomen and testes was noted, to which no significance was attributed. The pigmentation persisted until death, with what was definitely thought to be alternate increases and decreases of intensity. Six months after the onset of the above signs, it was noticed that male dogs of the neighborhood were being attracted to the house. It then also became obvious that these male dogs were being sexually attracted to Dog T and repeatedly attempted to mount him, whenever he was taken out. Dog T vigorously resisted such approaches. The dog's health remained good however, until one year after the onset of the skin pigmentation when it was noticed that the right testis was greatly enlarged to at least twice the size of the left. The right testis was hard and nontender. At this time slight difficulty with urination seemed apparent, but this symptom was not definite. There was also noticed marked enlargement of the nipples, particularly of the posterior ones. There was no weight loss, anorexia or apparent discomfort. Because it seemed that all the above findings must be related, Dog T was brought to the hospital for study.

Physical and laboratory findings

Examination of this animal revealed no evidence of weight loss and good general condition except for moderate alopecia, particularly of the abdomen and tail. There were numerous spots of black pigment in the skin of the abdomen and scrotum. The breasts were enlarged (Fig. 1). The right testis was stony hard and twice the size of the left. The prostate gland was enlarged to 2 or 3 times normal size.

The dog was placed in a metabolism cage and urine collected for a period of five days, 3800 cubic centimeters being obtained during this period. Analysis showed that this

PLATE 1

Hematoxylin and eosin stained sections of the testicular tumor (Zenker-formol fixation).



FIG. 3

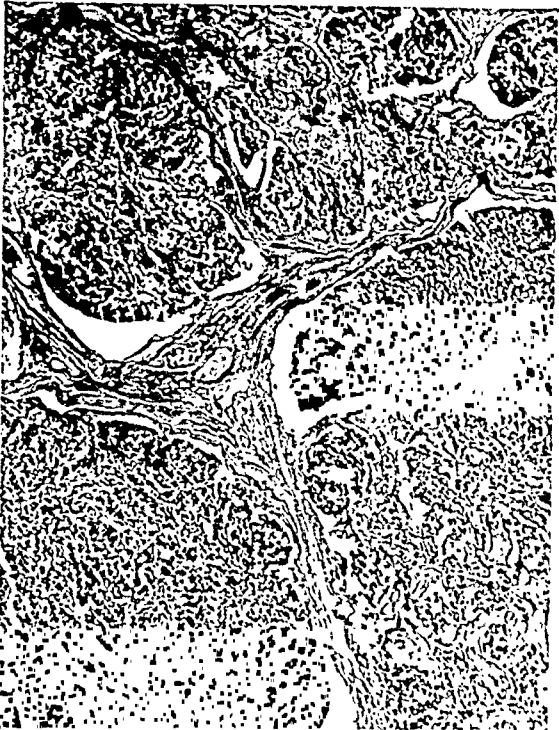


FIG. 4

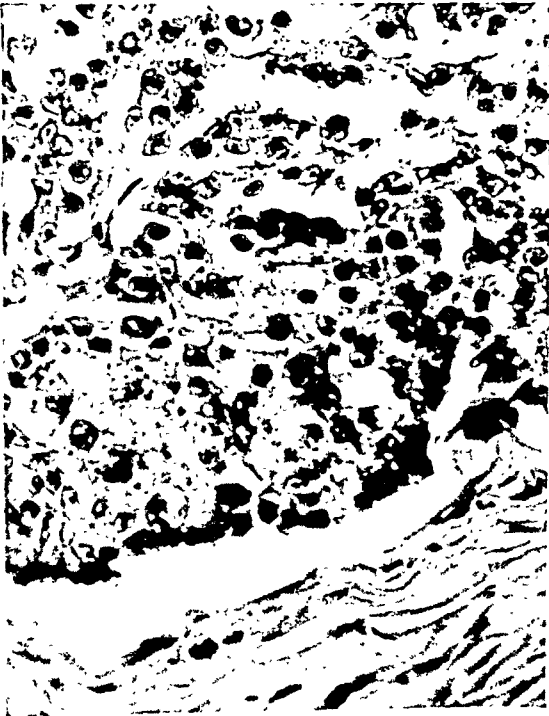


FIG. 5



FIG. 6

Microscopic examination

"Sections of the tumor show atrophic testis tubules compressed by a nonencapsulated tumor (Plate 1: Figs. 3 and 4). There is a tubular arrangement in some areas although definite lumina are seldom present. The appearance is occasionally that of solid testicular tubules, but most regions show large nodules and sheets of cells (Fig. 5). The tumor cells often present a palisading effect with their long axes perpendicular to the basement membranes of the tubules or to connective tissue strands and blood vessels of the solid portions. In some areas, the tumor is scattered through atrophic testis, and here the appearance is that of either local infiltration inside intact testis tubules or of the origin



FIG. 2. Hemisection of the tumor. The tumor bulges from the cut surface. It is arranged in lobules with areas of hemorrhage and softening. It is grossly circumscribed, markedly compressing the surrounding testicular tissue.

of the tumor within the tubules themselves (Fig. 6). The nuclei of the tumor are large and vesicular with prominent nucleoli. Mitotic figures are rare. The cytoplasm is pale, scanty and forms, in some areas, a syncytium. Fat stains show abundant fat droplets, large and small, in the tumor cells as well as in the connective tissue immediately surrounding the smaller tubules (Fig. 7). Leydig cells are not seen.

"The tumor in the periaortic nodes is composed of large sheets of similar cells which contain much less lipid.

"The prostate reveals striking squamous metaplasia (Fig. 8). The breasts show marked cystic hyperplasia, giving a 'thyroid' appearance to the low power view (Fig. 9). The left testicle shows atrophy with but few Leydig cells. Other sections are normal."

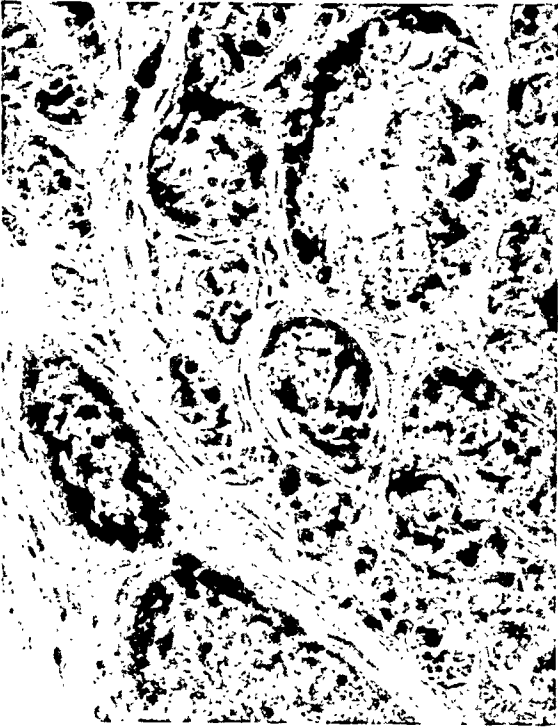


FIG. 7. ($\times 300$) Sudan stained frozen section of the main tumor mass. There is considerable fat in the tumor cells as well as in the connective tissue immediately surrounding the tumor masses, as seen in low center of figure.

FIG. 8. ($\times 300$) Hematoxylin and eosin stained section of prostate shows clearly the squamous metaplasia seen throughout all sections of the prostate.

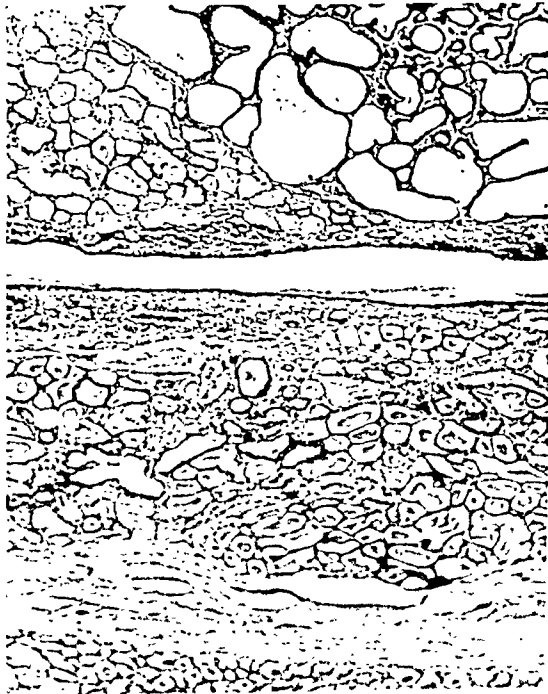


FIG. 9. ($\times 60$) Hematoxylin and eosin stained section through breast tissue in one of the enlarged nipples shows extreme dilatation of breast acini and ducts, surrounding a longitudinally cut mammary duct.

Analysis of the tumor for estrogen gave a value of 7.2 micrograms per kilogram expressed in terms of alpha-estradiol, a value between those reported by Huggins for these tumors.

In summary, a sudanophilic tumor with a high content of estrogen is seen to bear resemblance to those described in dogs as adenocarcinomata, "tubular adenomas" and as "Sertoli cell tumors." Squamous metaplasia of the prostate epithelium and cystic hyperplasia of the breast, similar to that found in dogs experimentally given estrogen is present. This tumor, described as benign in dogs, has here followed a prolonged course but has been found to have metastasized to abdominal lymph nodes.

DISCUSSION

Let us now turn to the human for evidence of estrogen production by the testis. In 1942 Witschi and Mengert in a study of two "sisters," examples of genetic intersex, presented evidence supporting their contention that "the sustentacular cells of the seminal tubules were the producers of female sex hormones which predominated in the hermaphrodites (the two "sisters") prior to castration." These two "sisters" observed by Witschi were almost identical in anatomic status. They were of almost equal age, 24 and 26 years, respectively. They had been raised as girls, possessed feminine voices and orientation, had well-developed breasts and a straight-topped female escutcheon of pubic hair. Their skin was smooth, and facial skin free of beard. However, their sex organs were predominantly male in character. Each had a bifid scrotum with two well-developed testes which on biopsy showed the tubular appearance of testis. Before surgery, urinary hormonal studies made in one of them showed moderate to normal estrogenic activity and only slight androgenic and gonadotropic activity. Three and one-half years after castration, urinary estrogen had decreased markedly and gonadotropic activity had increased to 100 rat units per day. At the time of removal of the scrotal gonads, exploratory laparotomy revealed no female pelvic organs. Sections of the scrotal gonads were said to show that the seminal tubules contained "an abundance of sustentacular cells of Sertoli." Maturation of sperm, to and beyond the stage of primary spermatocytes, appeared only rarely. No evidence of ovarian tissue could be seen.

FIG. 3. ($\times 60$) The low power photomicrograph shows a suggestively tubular pattern. In spite of prompt fixation and slow dehydration, considerable shrinkage unfortunately occurred.

FIG. 4. ($\times 60$) Other areas of the primary tumor and most of the metastatic tumor show more solid sheets of cells separated by acellular connective tissue strands.

FIG. 5. ($\times 500$) Cellular detail of the more solid portions of the tumor.

FIG. 6. ($\times 300$) Atrophic testicular tubules, adjacent to the tumor, surround a tubule replaced by tumor cells although the basement membrane remains intact. This probably represents invasion from the main tumor mass although multicentric origin of such tumors has been postulated on the basis of such a finding.

reduction in the size of the breasts. The tumor was considered an interstitial cell tumor. Powell (25) mentions a benign tumor, questionably an interstitial cell tumor, in which an excess of estrin was found in the urine. Östergaard (26) recently reported a case of a 28-year-old man with gynecomastia and increased estrin in the urine without excretion of gonadotropin. No changes in sexual potentia occurred. After removal of the benign testicular tumor, the urinary estrogens fell and the gynecomastia subsided. The tumor was thought to represent an adrenal rest tumor.

Finally, Teilum (27,28) reported a case of a 58-year-old man with gynecomastia, impotence, increased urinary estrogens and a sudanophilic tubular adenoma of the testes resembling rare types of ovarian tumors mentioned by Pick and considered to be a variety of an arrhenoblastoma. Photomicrographs of this tumor bear close resemblance to the tumors described here in dogs.

In 1947 Castillo (29) and his associates presented evidence for a syndrome "produced by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells." This syndrome is based on a series of 5 patients, aged 22 to 36 years, all of whom complained of sterility. These patients were normal on physical examination except for bilaterally small testes which on section were interpreted as showing small seminiferous tubules with normal basement membranes and Sertoli cells but with "complete" absence of germinal epithelium. Leydig cells appeared normal. Of primary interest to the discussion of the moment is that these individuals excreted normal amounts of follicle-stimulating hormone. Castillo and his co-workers suggest that the Sertoli cells produce a substance similar to estrogen, which regulates the production of the follicle-stimulating hormone of the pituitary. Here, as in the case of the next syndrome to be described, disease had produced a specific lesion of the germinal epithelium without apparent damage to either the Sertoli cells or cells of Leydig.

In considering testicular hormones other than masculinizing ones, mention of a second hormone commonly brings to mind a substance known as "inhibin." Based on experimental findings, Mottram and Cramer (30) in 1923 were the first to suggest that the germinal epithelium of the testis secretes a hormone distinct and independent from that of the Leydig cells. Support for this concept has come from the investigations of Martins and Rocha (31), McCullagh and Walsh (32), and Vidgoff and associates (33), as well as others. These authors have postulated that the germinal epithelium secretes a water-soluble hormone which functions in the inhibitory action of the testis on the pituitary.

In 1942 Klinefelter, Reifstein and Albright (34) presented evidence

Estrogen-producing testicular tumors occurring in dogs, such as in our case, Huggins' and others, must however, be excessively rare in humans. This is particularly true if masculine pseudohermaphrodite patients be excluded. Similar tumors without endocrine disturbances are also rarely reported.

In 1905, Pick (16) described both single and multiple adenomas in ectopic testes of masculine pseudohermaphrodites. These were called "adenoma tubulare testicularii ovarii" because of their similarity to ovarian tumors in both 'normal' women with menstrual difficulties, and in women with associated masculinization. This type of tumor has been considered as arising from Sertoli cells, being composed of cylindrical cells with palisading nuclei and cytoplasmic syncytia arranged in a tubular pattern, although other theories have been entertained. Chevassu (17) in 1906 found 3 of 128 testicular tumors to be tubular adenomas of either Sertoli cell or spermatogonia origin. Ewing (18) described 2 "pure adenomas" in cases of undescended testes and another in a pseudohermaphrodite. The microscopic picture described appears similar to that seen in dogs. Monaschkin (19) reported a case of gynecomastia occurring in an otherwise normal male but it is difficult to ascertain from either the pictures or the description whether this tumor could be considered an adenoma of the tubules or whether it was an interstitial cell or adrenal rest tumor. Rea (20) noted a case of a "carcinoma" of the undescended testes of a pseudohermaphrodite and considered that there were 51 cases of pseudohermaphrodites with ectopic testicular tumors, presumably adenomas, reported in the literature.

Engle and associates (21) in reporting a follow-up on a case of true hermaphroditism, who had been made "male," described the development of breasts at puberty even though the female elements had been removed four years before. Their photographs of the sections of the testis biopsy specimen reveal seminiferous tubules packed with Sertoli cells, which look much like the tumor described here.

Pace and Cabot (22) described 3 cases, taken from 24 specimens of undescended testis removed for repair of herniae, with "adenocarcinoma." The photomicrographs of these tumors show tubules of palisading cylindrical cells with intact basement membranes, similar to those described by Pick and others. Krückmann (23) described a case similar to Pick's in a masculine pseudohermaphrodite, with multiple tubular adenomas of the ectopic testes, absence of ovaries and marked gynecomastia. Hunt and Budd (24) reported a case of a 42-year-old man with gynecomastia, loss of libido, and a positive Aschheim-Zondek test but with a benign testicular tumor showing on analysis 20 mouse units of estrin in 1.73 grams of tumor tissue. Following orchidectomy, there was return of libido and a

nation of testicular biopsy specimens in these individuals showed lesions involving the tubular elements. In the advanced stages, hyalinization of all tubular elements was noted (*both* the germinal epithelium and Sertoli cell elements) but there was no destruction of the Leydig cells. These authors postulated a "dual hormone theory of testis physiology" suggesting that the second hormone—the hormone of the germinal epithelium—is "inhibin" and that "inhibin" is analogous and probably very similar to estrin.

In a comparison of these two syndromes, that of Castillo and that of Klinefelter, the specific difference lies in the presence of Sertoli cells in the former. A hormone produced by Sertoli cells and having a pituitary-inhibiting action would explain normal values for follicle-stimulating hormone observed in the Castillo syndrome. Absence of Sertoli cells and their secretion, in the syndrome described by Klinefelter, would explain the high titer of follicle-stimulating hormone observed in his patients (Table 1).

Certainly many of the actions of estrogens are similar to those reported for "inhibin." To enumerate only a few, estrogens inhibit the increased production of gonadotropins by the pituitary of the castrate and have been observed to cause a regression of the prostate and seminal vesicles of the normal rat. Both of these actions have been reported for "inhibin."

Evidence against a second hormone of the testis, whether it is estrin or "inhibin," is based on the observation that an androgen, such as testosterone, will inhibit gonadotropin production by the pituitary. There is little doubt as to the accuracy of this observation but question has been raised as to whether testosterone will effectively inhibit pituitary gonadotropin secretion when given in physiologic amounts. In this regard the observations of Howard (35) are of interest. He observed that effective pituitary inhibition in the human could be obtained with much smaller amounts of estrogen than of androgen. Specifically, in a castrate male, 1.0 milligram of diethylstilbestrol every two days was effective in the control of hot flashes and caused a drop in urinary gonadotropins from above 96 to less than 6 mouse units per day; 50 milligrams of testosterone propionate every two days was not effective in reducing gonadotropins to 52 mouse units per day, the upper limit of the normal range.

SUMMARY

Evidence is presented to substantiate the findings of others that, in the dog, feminizing testicular tumors exist which appear to be composed of Sertoli cells, are rich in lipids, and produce considerable quantities of estrogen. This evidence combined with other studies in humans in which estrogen production appears to be related to the presence of Sertoli cells,

TABLE I*

TABULATION OF THE DIFFERENCES BETWEEN THE KLINEFELTER, REIFENSTEIN AND ALBRIGHT SYNDROME (1942), EUNUCHOIDISM WITH INCREASED F.S.H. EXCRETION, THE CASTRATION SYNDROME, AND THE SYNDROME HERE DESCRIBED

	No. 1 Klinefelter, Reifenstein, and Albright (1942)	No. 2 Eunuchoidism with increased F.S.H.	No. 3 Castrates	No. 4 Syndrome here presented
Clinical manifestations of hypogonadism	absent or scarce	present	present	absent
Gynecomastia	present	frequently present	frequently present	absent
17-ketosteroids	normal or subnormal	reduced	reduced (usually)	reduced
F.S.H.	increased	increased	increased	normal
Azoospermia	present	present	present	present
Sertoli cells	destroyed	absent or atrophied	absent	present
Germinal epithelium	intense lesion	absent or atrophied	absent	absent
Walls of the tubules	hyalinized	absent or atrophied	absent	normal
Leydig cells	apparent hyperplasia	absent	absent	apparent hyperplasia
Testis size	small	absent or atrophied	absent	small

* Reproduced in its entirety from the article by del Castillo, Trabucco and de la Balze (29).

for the existence of a new syndrome. This syndrome, based on a study of 9 males, aged 17 to 38 years, is described as beginning in adolescence and is characterized by the findings of bilateral gynecomastia, small testes, aspermatogenesis and increased urinary gonadotropin. Histologic exami-

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forms the basis for the postulate that the second hormone of the testis is an estrogen produced by the sustentacular cells of Sertoli.

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In order to insure adequate urine volumes, the subjects ingested ample amounts of fluid starting two hours before and continuing until six hours after ACTH administration. Inclusive of the volume infused during this period, M.L. had a total fluid intake of 1800 ml.; M.J., 1200 ml.

Two preparations of ACTH¹ were employed. The properties of these products, presented in Table 1, show that they are potent adrenocorticotropins which contain only traces of other pituitary hormone activities. M.L. received 240 mg. of one preparation (37-KE), an amount equivalent

TABLE 1. BIOLOGIC PROPERTIES AND DOSES OF THE ACTH PREPARATIONS

	<i>Preparation 37-KE</i>	<i>Preparation 42-B(G-59703)</i>
ACTH activity	—41.5 ± 12 per cent of Armour standard La-1-A*	—16 ± 4 per cent of Armour standard La-1-A
Posterior pituitary hormones—oxytocic—0.0025 U.S.P. unit per mg.		—0.0036 unit per mg.
	—pressor—0.005 unit per mg.	—0.0045 unit per mg.
Prolactin	—0.5 unit per mg.	—1 unit per mg.
Growth hormone	—negligible	—negligible
Gonadotropins	—2.0 Collip units per mg.	—1.5 Collip units per mg.
Thyrotropin	—0.037 ± 0.06 Evans chick unit per mg.	— ?
Moisture	—5.96 per cent	— ?
Administered to	—M.L.	—M.J.
Total amount infused	—240 mg.	—330 mg.
Total ACTH activity infused equivalent to La-1-A	—100 mg.	—50 mg.

* Highly potent Armour ACTH standard.

in ACTH activity to 100 mg. of a purified standard (La-1-A, Armour); M.J. received 330 mg. of the other preparation (42-B), an amount equivalent in ACTH activity to 50 mg. of the same standard. Despite the large doses administered, evidence of pressor activity was not observed in either subject; inhibition of diuresis, a most sensitive index of posterior pituitary activity,² occurred in M.L. only.

Before the infusion of ACTH was started, both subjects were examined for their sensitivity to the ACTH preparations by ophthalmic and intradermal tests, and gave negative responses. Moreover, when reexamined by the same tests six weeks after the experiment, neither subject showed any evidence of acquired sensitivity.

¹ Kindly supplied by Dr. John Mote of the Armour Laboratories.

² Quantities as small as 0.5 pressor unit injected intramuscularly will produce a marked inhibition of water diuresis (4).

METABOLIC ACTIONS AND FATE OF INTRAVENOUSLY ADMINISTERED ADRENOCORTICOTROPIC* HORMONE IN MAN†§

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THE metabolic alterations following the administration of pituitary adrenocorticotrophic hormone (ACTH) by the intramuscular or subcutaneous route have been reported by a number of investigators (1, 2, 3). The present study concerns the metabolic actions and fate of single large doses of ACTH injected intravenously. Special attention has been paid to the temporal relationship between the dynamic changes which occurred in certain constituents of the body fluids and the titer of tropic hormone in the plasma. Renal excretion of the administered hormone has also been studied.

MATERIAL AND METHODS

The 2 subjects of these experiments were healthy male medical students; M.L. was 26 years old and weighed 84 Kg., and M.J. was 29 years old and weighed 89 Kg. They were on a controlled diet and lived in the metabolic ward of the Department of Medicine for a number of days before and during the hormone studies. Subject M.L. consumed 3100 calories per day which included 360 Gm. of carbohydrate. Subject M.J. received 2450 calories per day which included 260 Gm. of carbohydrate. Breakfast was withheld from both subjects on the morning of the experiments, but a meal was given six hours following the beginning of hormone administration. For M.L. this meal contained 100 Gm. of carbohydrate; for M.J., 80 Gm. of carbohydrate. Both subjects were again fed during the tenth hour. This meal contained 150 Gm. of carbohydrate for M.L. and 110 Gm. for M.J.

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* The term *tropin* is the form used by the Journal. The authors favor *trophin*.

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§ Most of the data appearing in this paper were presented before the thirtieth annual meeting of the Association for the Study of Internal Secretions, Chicago, June 18, 1948.

TABLE 2. PLASMA TITERS OF INTRAVENOUSLY ADMINISTERED ACTH

Subject	Time in hours following beginning of ACTH infusion	Plasma injected into test rats (ml. per 100 Gm. body weight)	No. of rats	Response as measured by reduction in ascorbic acid (mg. per 100 Gm. adrenal tissue,* av. & std. error)	Estimate of concentration of ACTH in plasma (micrograms per 100 ml.)
M.L.	0	2	4	16 ± 3	<10
	End of one hour infusion period	0.1	4	75 ± 4	250-1000
		0.5	3	124 ± 6	
	2	1.0	4	53 ± 11	25-100
	3	2	3	14 ± 6	<10
	6	2	3	24 ± 11	<10
	9	2	3	-5 ± 5†	<10
	12	2	4	14 ± 7	<10
	24	1	2	-12†	<10
M.J.	0	2	5	9 ± 6	<10
	End of 30-minute infusion period	0.5	4	111 ± 15	100-400
		2	4	131 ± 17	
	1	0.5	4	38 ± 4	50-150
		2	3	111 ± 5	
	1.5	2	4	53 ± 16	25-100
	2	2	5	31 ± 8	10-40
	3	2	7	18 ± 10	<10
	6	2	5	17 ± 7	<10

* Left adrenal removed as control. Plasma injected via tail vein. One hour later right adrenal removed. Response expressed as difference in concentration of ascorbic acid between left and right glands.

† Negative sign means concentration of ascorbic acid in right gland greater than that in left.

The hormone preparations were infused in 200 ml. of physiologic saline. For the first subject, M.L., the time of infusion was one hour, but approximately 75 per cent of the total amount was given at a constant rate during the last half-hour. In the case of M.J., the entire 200 ml. was infused at a constant rate over a period of one-half hour.

For ACTH determinations, plasma and urine samples were shell frozen ready for lyophilization within a period of one hour following their procurement. Studies of two of the authors (G. S. and T. W. B.) have shown that the procedure is effective for preserving ACTH activity. ACTH activity was determined by the adrenal ascorbic acid-depletion method of Sayers, Sayers and Woodbury (5).

Blood sugar was determined by the colorimetric copper method of Nelson (6) and Somogyi (7); serum phosphate, by the technic of Fiske and SubbaRow (8). Urinary nitrogen was measured by nesslerization following digestion of the sample.

The hematologic studies were conducted by routine clinical methods except for the eosinophil counts which were determined according to the method described by Forsham *et al.* (2).

Serum proteins were characterized by electrophoretic studies in a Tiselius apparatus equipped with the Longworth schlieren scanning device. The measurements were conducted at 1° C after equilibration for forty-eight hours with diethyl barbiturate (veronal) buffer at pH 8.5 to 8.6 and at an ionic strength of 0.1. Photographs were taken of the descending boundaries only. The protein concentration in the cell was 1.5 Gm. per 100 ml.

Antistreptolysin O was measured by the method of Todd (9) as modified by Lancefield (10). Isoagglutinins, agglutinins to typhoid H and O antigens, and diphtheria antitoxin were estimated by procedures recommended by Enders (11). In subject M.L. the serum gamma globulin was measured immunologically by the quantitative precipitin technic (12). The antiserum for this determination was prepared by immunizing rabbits to a highly purified human gamma globulin.

Peptidase assays on samples of serum were performed within twenty-four hours of the withdrawal of the blood specimens. The measurements were made by the titration of liberated carboxyl groups (13). The hydrolysis of the following was studied: glycylglycine (glycylglycine dipeptidase (14)), triglycine (tripeptidase (15, 16)), glycyl-L-proline (prolidase (16)), and L-leucinamide (leucine aminopeptidase (15, 17)).

Uric acid was determined by Archibald's modification of the technic of Kern and Stransky (18) as described by Forsham *et al.* (2). The Jaffe reaction (19) was applied to the determination of creatinine and creatine.

Sodium and potassium in plasma and urine were measured in a flame

in the plasma of M.J. correspond to the smaller dose of ACTH which was given to this subject.

Samples of urine were obtained at various times during the course of the experiment. The concentration of hormone in the urine was never greater than 10 micrograms per 100 ml. of urine. It may be estimated that in each subject less than 200 micrograms of ACTH appeared in the urine during the 24-hour period following hormone administration. Unless the hormone is present in the urine in some inactive form, renal excretion can be disregarded as a significant factor in the elimination of ACTH from the body.

The very rapid disappearance of the administered hormone from circulating plasma may be due to inactivation and/or rapid diffusion of the

TABLE 3. ALTERATIONS IN BODY FLUID CONSTITUENTS ASSOCIATED WITH GLUCONEOGENESIS

Subject	Time in hours following beginning of ACTH infusion	Blood sugar, mg. per 100 ml.	Serum phosphate, mg. per 100 ml.	Urine collection periods*	Urinary phosphate excretion, mg. per mg. creatinine	Urinary nitrogen, mg. N per mg. creatinine per min.
M.L.	0	98	4.8	-1- 0	—	6.2
	1	118	—	0- 1	—	4.9
	2	96	4.1	1- 2	—	6.3
	3	105	4.1	2- 3	—	6.8
	4	103	3.9	3- 4	—	7.6
	5	112	4.5	4- 5	—	9.7
	6 (Meal 6.0)	96	4.8	5- 6	—	5.8
	9 (Meal 9.5)	205	4.1	6- 9	—	5.7
	12	103	4.6	9-12	—	4.8
	24	100	4.4	12-24	—	4.8
M.J.	0	82	3.5	control 24 hours	0.39	6.7
	2	78	3.5	0- 3	0.26	7.5
	3	93	4.0	3- 6	0.37	16.3
	6 (Meal 6.0)	97	3.9			
	6.7	162	3.6	6-12	0.39	7.7
	7.1	197	3.3			
	7.9 (Meal 9.5)	183	4.0			
	12	104	—	12-24	0.54	7.5
	24	85	—			

* The numbers in this column indicate hours following the beginning of ACTH infusion.

photometer with an internal lithium standard; chloride was determined by the method of Schales and Schales (20) with precautions regarding pH (21); plasma pH was measured on a standardized glass electrode and the CO₂ content of the plasma by the manometric technic of Van Slyke and Neill (22). Blood for the above analyses was obtained from the antecubital vein without venostasis and without exposure of the blood to air.

Urinary 17-ketosteroids were measured by the technic of Callow (23) corrected for interfering chromogens as outlined by Talbot (24). Neutral-lipid reducing substances in the urine were determined by the method of Heard, Sobel and Venning (25) which measures all steroids with a C₂₀ carbonyl group, thus including desoxycorticosterone and the C₁₁ oxygenated cortical hormones.

RESULTS

Clinical observations. Untoward reaction to the hormone was minimal. M.L. had a mild, shaking chill of undetermined origin, which began 75 minutes after the start of the infusion and lasted for twenty minutes. This subject's temperature was 97.5° F. at the end of the chill and one hour later had risen to 99.4° F. No significant alteration in blood pressure occurred during the course of the experiment in either subject. The post-infusion course of M.J. was entirely uneventful.

Fate of adrenocorticotropin. The ACTH content of body fluids is expressed in terms of the amount of the standard which would produce an equivalent response in the bioassay animal. Assays on control plasma and urine samples taken from both subjects before the experiment indicated that ACTH was not present in detectable amounts, *i.e.*, there was less than 10 micrograms per 100 ml.³

The data in Table 2 show that the concentration of hormone in the plasma of M.L. at the end of the infusion period was equal to 500 (250 to 1000) micrograms⁴ per 100 ml.; two hours after the beginning of the infusion it was 50 (25 to 100) micrograms⁴ per 100 ml. After the second hour, the amount of hormone in the plasma was indistinguishable from pre-infusion values. As shown in Table 2 the rate of disappearance of the hormone from the plasma of M.J. was similar to that of the first subject. The lower titers

³ This estimate of hormone content is based upon the sensitivity of the assay method. Quantities as small as 0.2 microgram of a highly purified preparation of ACTH can be detected. Hence, when 2.0 ml. of plasma injected into the test animal give either a questionably significant or zero response, then 100 ml. of this plasma will contain an amount of hormone activity less than that of 10 micrograms of the standard (La-1-A).

⁴ These titers are expressed as a range which indicates the error of the estimate of the potency. For example, the sample of plasma taken from M.L. at the end of the infusion was estimated to contain 500 micrograms per 100 ml., with a probable error of -50 to +100 per cent.

second large carbohydrate meal in the interval. Both subjects showed increased total urinary nitrogen excretion in the early period after ACTH administration, a fact consistent with the view that carbohydrate was being formed at the expense of tissue protein. These metabolic alterations are typical of the well-known gluconeogenic action of cortical steroids with an oxygen on C-11. The results confirm and extend the findings of Forsham *et al.* (2) who showed somewhat comparable changes following chronic administration of ACTH. Thus the metabolic evidence suggests that ACTH brings about an increased secretion of steroids with an oxygen on C-11 from the adrenal cortex.

Serum and urinary inorganic phosphate changes were not sufficiently definite or consistent to warrant any conclusions based on them.

Formed elements of the blood. In both subjects the total white blood cell count, which was initially normal, became elevated because of an increase in the number of circulating neutrophils (Table 4). In contrast, the number of circulating lymphocytes markedly decreased and within a period of two hours reached a minimal level which persisted through the fourth to sixth hours. By the ninth hour the lymphocyte count was either normal or above normal. In both subjects, the number of circulating eosinophils fell markedly and reached a minimum at five to seven hours after administration of hormone. By the twelfth hour the number of eosinophils was either normal or approaching normal and by the twenty-fourth hour had definitely returned to normal.

In the case of M.L., despite the withdrawal of more than 500 ml. of blood, the volume of packed red blood cells remained unchanged over a period of twenty-four hours (Table 4). The volume of packed red blood cells of M.J. from whom 600 ml. of blood was withdrawn remained relatively constant during the first six hours of the experiment and then declined from 52 to 38 ml. per 100 ml. of blood at the end of twenty-four hours.

In subject M.L., there was a suggestive increase in the erythrocyte sedimentation rate at the third hour. For this reason serial estimations of plasma fibrinogen (method of Cullen and Van Slyke (27)) were made on subject M.J. However, no change occurred in the erythrocyte sedimentation rate or the fibrinogen concentration of the plasma of subject M.J. It is probable that the increased sedimentation rate in M.L. was related to the chill and fever which he experienced rather than to an effect of ACTH mediated by the adrenal cortex.

Serum iron and copper were determined on samples of blood taken immediately before, and at six and seven hours following, the administration of ACTH. No significant alteration occurred in the serum concentrations of these elements.

The changes in the cellular elements of the blood confirm the experi-

TABLE 4. HEMATOLOGIC CHANGES

Subject	Time in hours following beginning of ACTH infusion	Total white count, cells per cu. mm.	Neutrophils, cells per cu. mm.	Lymphocytes, cells per cu. mm.	Eosinophils, cells per cu. mm.	Platelets per cu. mm.	Hematocrit, ml. packed r.b.c. per 100 ml. blood	Erythrocyte sedimentation rate, cu. mm. per hr.
M.L.	0	4,950	1,930	2,520	57	—	45	2.5
	1	3,250	1,720	1,200	8	—	46	8
	2	5,950	4,450	475	22	—	46	—
	3	9,800	7,120	784	0	—	45	20
	4	11,050	7,820	884	19	—	46	3
	5	—	—	—	3	—	46	6
	6	9,350	5,800	2,710	3	—	46	3
	9	9,950	6,760	2,490	10	—	46	—
	12	—	—	—	61	—	46	5
	24	4,500	2,120	1,710	67	—	45	6
M.J.	0	9,150	5,120	3,610	200	220,000	52	0
	2	8,100	4,860	1,540	108	210,000	48	0
	3	9,650	6,380	1,540	97	140,000	49	2
	6	11,450	7,900	1,490	11	125,000	49	2
	7	—	—	—	8	—	—	—
	12	13,650	6,410	5,740	100	—	41	—
	24	9,000	3,780	3,440	186	270,000	38	2

hormone into the tissues. Because ACTH could conceivably be excreted in the urine in a conjugated inactive form and since the activity of certain preparations of ACTH can be enhanced by acid treatment,⁵ the urine of subject M.L. was acidified to pH 1.0; however, no increase in ACTH activity was found. In contrast to ACTH, gonadotropin, after a single injection in the human, is excreted in the urine in appreciable quantities for a number of days (26). The ACTH preparations employed in the present study were isolated from hog pituitaries; one cannot be certain that the ACTH elaborated by the human pituitary is subject to the same fate as the porcine hormone.

Blood sugar, serum phosphate, and urinary phosphate and nitrogen. No significant change occurred in the fasting blood glucose (Table 3). Following a meal of 80–100 Gm. of carbohydrate, both individuals had glycosuria exceeding two per cent and abnormally high blood glucose levels. Glucose was no longer detectable in the 9–12 hour sample of urine of both subjects and blood sugar values returned to normal in spite of the ingestion of a

⁵ Unpublished data of T. W. B. and G. S.

TABLE 5. EFFECT OF ACTH ON SERUM PEPTIDASES

The substrates (0.05 M) were glycylglycine (gg) in the presence of 0.001 M Co^{++} , glycylglycylglycine (ggg), *L*-leucinamide (la) in the presence of 0.001 M Mn^{++} , and glycyl-*L*-proline (gp) in the presence of 0.001 M Mn^{++} . The solutions were buffered with 0.025 M veronal at pH 7.8. The total volume of each mixture was 2.5 ml., and hydrolysis was determined on 0.2 ml. samples by the titration of liberated carboxyl groups by the method of Grassmann and Heyde (13). The results are expressed as $K \times 10^4$, the first order velocity constant for 0.2 ml. serum per ml. of reaction mixture. Those values which appear to differ significantly from the control values are in bold-face.

Subject	Time in hours following beginning of ACTH infusion	gg	ggg	la	gp
M.L.	0	2.2	3.1	0.3	4.6
	3	3.5	3.9	0.5	3.4
	6	4.9	4.2	0.3	4.3
	12	1.9	2.7	0.4	4.4
	24	2.0	3.3	0.8	3.5
	48	2.0	2.7	0.7	3.8
	96	2.6	2.9	0.4	2.9
M.J.	-24	4.5	3.0	0.5	2.6
	0	4.5	3.7	0.4	3.4
	3	4.5	3.3	0.5	4.0
	6	4.8	6.0	0.5	2.8
	24	4.1	4.1	0.5	3.0

No antibodies to the H and O antigens could be demonstrated in his serum taken immediately prior to or at intervals during the twenty-four hours following ACTH injection. The isoagglutinin titer of this subject (Group O) to group A and B cells did not change throughout the course of the experiment. The control diphtheria antitoxin level of 0.6 unit in his serum, as measured by the rabbit skin test, did not change significantly during the ninety-six hours following ACTH administration.

The failure of the gamma globulins and serum antibodies to rise in the normal human following administration of ACTH is in contrast to the experimental results of White and Dougherty (29) in rabbits. However, our results confirm the negative findings of Forsham *et al.* (2) and of Herbert and deVries (30) in human subjects. The evidence available at present, from experiments in which large doses of ACTH are given, appears to indicate that in the human the secretory activity of the adrenal cortex can play no more than a minor role in the mobilization of antibodies from tissue stores. It is possible that only a meager quantity of antibodies is stored in the human, so that no significant increase in their serum level could be expected following administration of ACTH.

ments of White and Dougherty in animals (28) and of Forsham *et al.* (2) in human subjects. The elevation of the number of circulating neutrophils is probably not a result of activation of the adrenal cortex, since this same effect can be obtained with ACTH in adrenalectomized animals (28) and in patients with Addison's disease (2). Because ACTH does not produce a lymphocytopenia in adrenalectomized animals (28) or in patients with adrenal insufficiency (2), it is fairly certain that the lymphocytopenia is mediated by the adrenal cortex, in particular, by the action of cortical steroids which have gluconeogenic properties. Similarly, the decrease in the number of circulating eosinophils which follows administration of ACTH has been shown by Forsham and associates (2) to depend upon the presence of functioning adrenal cortical tissue.

Serum proteins. Samples of blood were taken from M.L. at 0, 3, 6, 12, 24, 48 and 96 hours. The electrophoretic distribution of serum proteins was normal at all these times. The $\alpha_2 + \alpha_1$ globulins were found to be 16 ± 1 per cent; $\beta_2 + \beta_1$, 11 ± 1 per cent; $\gamma_2 + \gamma_1$, 14 ± 2 per cent; and albumin, 59 ± 3 per cent. Samples of serum were taken from M.J. at 0, 3, 6, 12 and 24 hours. Again, distribution of the serum proteins was normal and remained unchanged throughout the entire series. Values obtained were as follows: $\alpha_2 + \alpha_1$, 11 ± 1 per cent; $\beta_2 + \beta_1$, 11 ± 1 per cent; $\gamma_2 + \gamma_1$, 14 ± 2 per cent; and albumin, 64 ± 1 per cent. It should be noted that although the α -globulin concentration differed markedly in the two subjects, there was no significant change in either case following injection of ACTH.

In patient M.L., the "gamma globulin," as estimated immunologically, ranged from 27 to 31 per cent of the total serum protein. The values for gamma globulin as estimated immunologically (12) are greatly in excess of the values obtained electrophoretically. The control value was 30 per cent and a maximum value of 31 per cent was attained at the third hour; thereafter, the value ranged between 27 and 28 per cent of the total serum protein, up to and including the ninety-sixth hour. Such alterations as were noted in this protein fraction after administration of ACTH do not appear significant.

Immune bodies. M.L. had an elevated antistreptolysin O titer (200 units) during the control period. The titer did not change appreciably in the serial estimations performed during the seventy-two hours after ACTH administration. Although this subject twice received typhoid vaccine in the previous three years, he had no demonstrable antibodies to H and O antigens during the control period or subsequent to ACTH injection.

M.J. also had an elevated antistreptolysin O titer (250 units) prior to injection of ACTH and showed no increase subsequently. This subject had received typhoid vaccine in the past three years and, in addition, received 1.0 ml. of typhoid vaccine subcutaneously five days before the experiment.

pared to the rates before injection of ACTH and subsequent to six hours. With gp, a slight increase which does not appear significant was evident at three hours, but the rate at the sixth hour returned to the control value. The ability to hydrolyze ggg showed a marked increase at six hours. Again, the level of leucine aminopeptidase activity did not show any change.

The above data confirm, at least in part, the observation by Holman and associates (31) that adrenal cortical stimulation causes a rise in the level of certain serum peptidases. In confirmation of their results with lgg, we found a small but apparently significant rise in tripeptide (ggg) hydrolysis. There was no detectable change in either of our subjects in leucine aminopeptidase or prolidase activity. However, in the serum of M.L., a fairly marked change occurred in gg dipeptidase activity, but only a slight change was found in the serum of M.J. The small and variable changes observed in the serum peptidases do not appear to be very striking, especially in comparison with the marked decrease observed in the circulating lymphocytes and eosinophils.

Serum uric acid and urinary uric acid, creatinine and creatine. The results are presented in Table 6. The most marked alteration in these constituents was the increased rate of excretion of uric acid. Values for uric acid in the urine are expressed as ratios of uric acid to creatinine. The peak effect appeared in the second hour. The result confirms the observations of Forsham and co-workers (2). The temporal association of lymphocytopenia and increased uric acid excretion suggests that lysis of lymphocytes may be the factor responsible for the increased output of uric acid in the urine. However, further analysis of the data suggests that two processes are occurring simultaneously. The serum uric acid concentration did not fall during the period of increased urinary excretion; indeed, it rose somewhat. This suggests that both increased uric acid production and increased urinary excretion took place, an interpretation which gains support from the fact that a phase of uric acid retention occurred after most of the other metabolic changes had disappeared.

Plasma pH and CO₂, and plasma and urinary sodium, potassium and chloride. In subject M.L., there was a definite elevation in plasma pH at the second hour; the pH returned to normal by the sixth hour (Table 7). Unfortunately, no studies were made in this subject to determine the CO₂ content of the plasma. The plasma of subject M.J. underwent no change from normal values for venous blood in pH, CO₂ content or pCO₂ up to the sixth hour of the experiment, at which time the observations were terminated.

Cluxton and associates (32) have shown that in certain patients with Cushing's syndrome there is an elevation of plasma pH and CO₂ content.

Serum peptidases. The activity of several serum peptidases was measured after ACTH injection in order to determine whether changes occurred in humans similar to those reported in mice by Holman, White, and Fruton (31). These investigators measured the activity of leucine aminopeptidase (substrate: *L*-leucinamide), and of a tripeptide-splitting enzyme (substrate: *L*-leucylglycylglycine) before and after the administration of cortical extracts and of ACTH. Cortical extracts significantly increased the level of both enzymes; ACTH increased only the tripeptidase level.

The conditions of the measurements and the results are given in Table 5. It is apparent that a definite increase in the hydrolytic activity of the serum of M.L. occurred at three and six hours, as measured with glycylglycine (gg) and glycylglycylglycine (ggg); the level of activity returned to normal by the twelfth hour. The rate of hydrolysis of glycylproline (gp) and of leucinamide were not significantly altered.

Small but insignificant changes in the rates of hydrolysis of gg and ggg by the sera of M.J. appear to have taken place at six hours when com-

TABLE 6. ALTERATIONS IN SERUM URIC ACID, URINE URIC ACID, CREATINE AND CREATININE

Subject	Urine collection periods*	Serum uric acid, mg./100 ml. at beginning of period	Urine creatinine, mg. excreted per minute	Urine uric acid, mg. excreted per minute	Ratio uric acid/creatinine	Per cent change in UA/C
M.L.	Control	3.9	1.18	0.43	0.36	
	0-1	—	1.11	0.58	0.53	+47
	1-2	3.7	1.08	0.71	0.66	+83
	2-3	3.7	1.45	0.81	0.56	+56
	3-4	4.0	1.18	0.48	0.41	+14
	4-5	4.0	0.97	0.32	0.33	-8
	5-6	4.0	1.25	0.44	0.35	-3
	6-9 (Meal 6.0)	5.0	1.30	0.70	0.54	+50
	9-12 (Meal 9.5)	4.0	1.43	0.31	0.22	-39
	12-24	3.7	1.30	0.32	0.25	-31
M.J.	Control	5.9	1.35	0.43	0.32	
	0-3	6.2	1.35	0.64	0.47	+47
	3-6	6.6	1.31	0.55	0.42	+31
	(Meal 6.0)					
	6-12 (Meal 9.5)	6.3	0.85†	0.15	0.18	-44
	12-24	6.1	1.36	0.28	0.20	-37

* The numbers in this column indicate hours following the beginning of ACTH infusion.

† This collection appears to be incomplete.

larger dose of pressor substance according to animal assay. Since pressor and antidiuretic activities usually parallel one another, it is surprising that there was no inhibition of water diuresis in M.J.

Both subjects were on a constant daily intake of sodium, potassium and chloride for a number of days before and after the administration of ACTH. M.L. ingested 196 mEq. of sodium and 102 mEq. of potassium per day. On the day of the experiment he received an additional 29 mEq. of sodium in the infusion fluid containing the ACTH. During the first six hours after

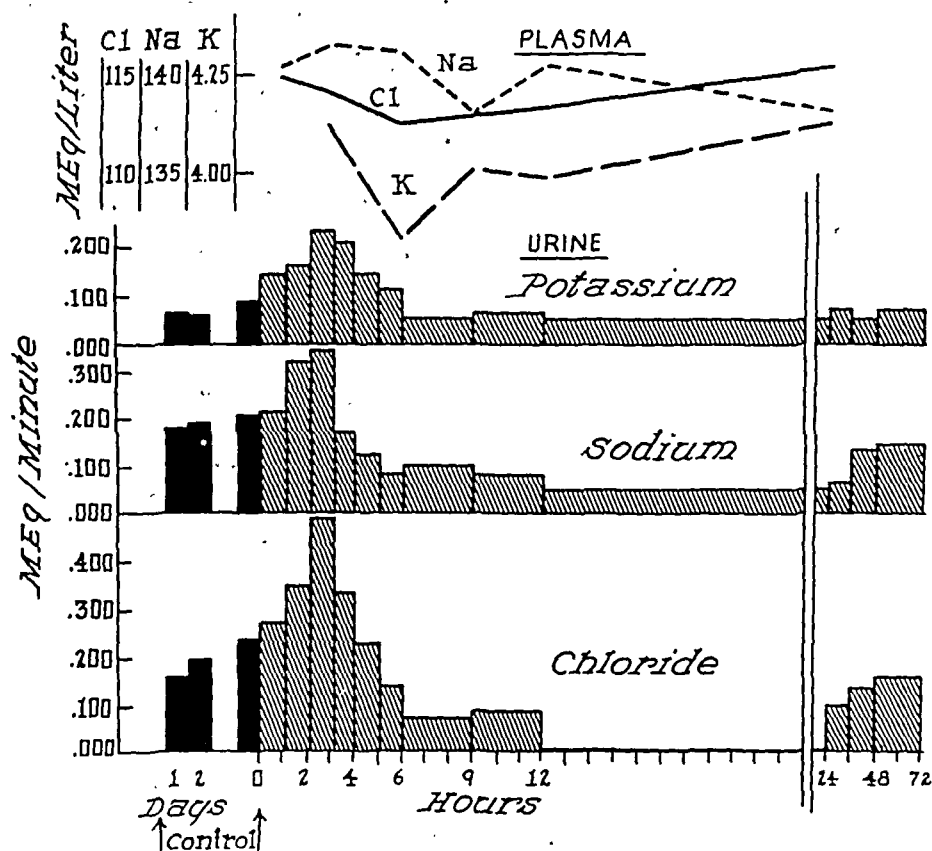


FIG. 1. Electrolyte studies on M. L.

ACTH administration 400 ml. of blood was withdrawn, which represents a loss of about 28 mEq. of sodium and 0.8 mEq. of potassium. An additional 150 ml. of blood was withdrawn between the sixth and eighteenth hours. Plasma concentrations of chloride and sodium for M.L. (Fig. 1) remained constant during the twenty-four hours following ACTH administration; plasma potassium was lowered at the sixth hour. During the first four hours after hormone administration there was an elevation in the rate of urinary excretion of chloride, sodium and potassium. From this time on, there was a marked retention of sodium and chloride which persisted at least to the thirty-sixth hour. When account is taken of all

A similar elevation in pH took place in subject M.L. However, no change in pH followed ACTH administration in subject M.J. and the pH change in M.L. was temporally associated with his chill and fever and disappeared before the other metabolic changes reached their peak. For these reasons, it seems likely that the alteration in pH was the result of hyperventilation accompanying the chill and fever and not a result of adrenal cortical stimulation by ACTH.

Both subjects were studied for an antidiuretic effect following ACTH administration. None was found in M.J. However, injection of ACTH

TABLE 7. ALTERATIONS IN pH AND CO₂ OF VENOUS PLASMA

Subject	Time in hours following beginning of ACTH infusion	pH	Bicarbonate, mM. per liter	pCO ₂ , mm. of mercury
M.L.	0	7.37		
	1	7.46		
	2 chill	7.60		
	3	7.53		
	6	7.33		
	9	7.38		
	12	7.33		
M.J.	0	7.31	27.3	56
	1	7.36	28.0	50
	2	7.31	27.9	57
	3	7.30	27.5	58
	6	7.36	25.9	49

(preparation 37-KE) inhibited a water diuresis in M.L. This subject drank 600 ml. of water between 7:30 a.m. and 8:10 a.m. and an additional 600 ml. between 9:00 a.m. and 12:15 p.m. on the day of ACTH administration. Two hundred ml. of 0.85 per cent sodium chloride solution containing the ACTH was infused between 9:10 a.m. and 10:00 a.m. Although diuresis had started (200 ml. of urine excreted between 7:30 a.m. and 9:00 a.m.), it was soon inhibited (360 ml., between 9:00 a.m. and 12:15 p.m.) and remained so until the third hour after injection of ACTH, when it reappeared. Between the third and sixth hours, 760 ml. of urine was excreted; 400 ml. of water was ingested during this same interval. The pressor activity of the two preparations of ACTH employed in these experiments is similar (Table 1), according to animal assay. However, since M.J. received a greater quantity of total solids he was injected with a correspondingly

value. From the sixth to the twelfth hour the rate of urinary potassium excretion was reduced, but not in proportion to the marked reduction in urinary excretion of sodium and chloride.

The transient sodiumphoresis and chloruresis in M.L. were associated in time with the inhibition of water diuresis in this subject. All three effects were probably due to the action of posterior pituitary antidiuretic principle, which contaminated the particular ACTH preparation. The observed reduction in plasma potassium, elevation in rate of urinary excretion of potassium, and retention of sodium and chloride are all changes which may reasonably be assigned to the action of desoxycorticosterone-like steroids elaborated by the adrenal cortex. Tissue breakdown as measured by the increased nitrogen excretion does not account for any significant part of potassium excreted in excess of normal during the experimental period. The alterations in potassium occurred at the peak of the other metabolic changes. However, the delay in the onset of sodium retention and its long persistence are not readily explained. Final conclusions regarding the effect of ACTH upon electrolyte balance must await the results of studies employing preparations in which the posterior pituitary principle is either absent or so reduced in amount that it exerts negligible physiologic actions. The preparation employed in M.J. did not inhibit water diuresis but it had pressor action by laboratory assay. The lack of parallelism between antidiuretic and pressor potency is unexpected.

Forsham and associates (2) demonstrated that daily administration of ACTH in humans resulted in marked retention of sodium and chloride and an increased urinary excretion of potassium, effects which may be attributed to the secretion of desoxycorticosterone-like steroids from the adrenal. However, in the first four hours after ACTH administration Forsham *et al.* noted an increase in the rate of urinary excretion of sodium and chloride as well as potassium. This same pattern of action was observed by us in M.L., given a preparation with antidiuretic potency, but was not seen in M.J., given one which did not inhibit diuresis. Forsham and co-workers consider the possibility that "the increased sodium, chloride, and potassium-creatinine ratios which occurred in the first four hours following ACTH administration reflect a predominant secretion of 11-17-oxysteroids as opposed to desoxycorticosterone-like factors under which circumstances an increase in potassium excretion would be accompanied by sodium and chloride retention".

Steroids. During the 24-hour period following ACTH administration, M.L. had an elevated urinary excretion of both the neutral-lipid reducing substances of Heard, Sobel and Venning (25) and the 17-ketosteroids (Table 8). In M.J., a more detailed temporal analysis was made. The most marked elevation in the excretion of both types of urinary steroids occurred during the first six hours after ACTH administration (Table 8).

sources of sodium gain and loss, there was an over-all retention of about 100 mEq. of sodium during the twenty-four hours following ACTH injection, as compared with the control periods. On the other hand, the rate of urinary excretion of potassium was elevated for the first six hours, returned to control levels by the seventh hour and remained there through the seventy-second hour, when observations were terminated.

M.J. ingested 127 mEq. of sodium and 97 mEq. of potassium per day and on the day of the experiment received an additional 29 mEq. of sodium

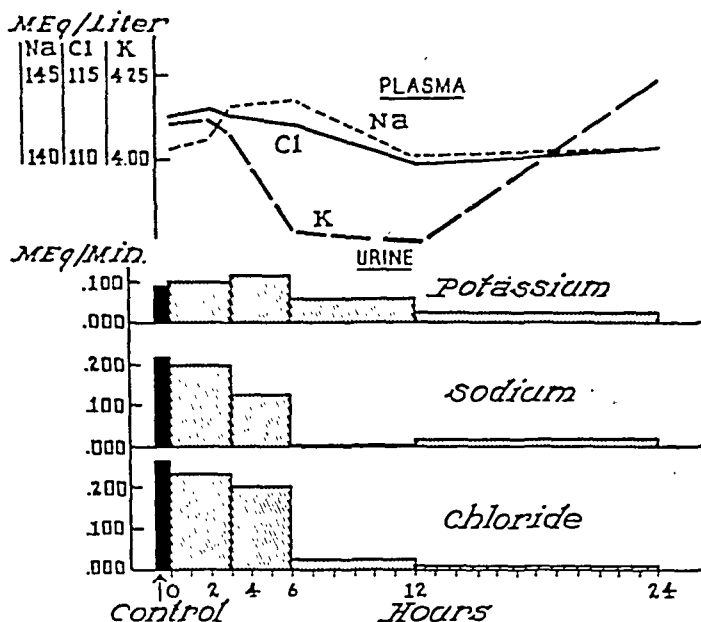


FIG. 2. Electrolyte studies on M. J.

in the infusion fluid containing the ACTH. About 29 mEq. of sodium and one mEq. of potassium were lost by removal of blood during the first six hours of the experiment. An additional 18 mEq. of sodium was withdrawn during the sixth to eighteenth hours. Plasma concentrations of sodium and chloride showed no significant change during the twenty-four hours after ACTH administration (Fig. 2). However, the plasma potassium level was depressed between the sixth and twelfth hours. The rate of urinary excretion of sodium and chloride was moderately depressed during the first six hours and markedly decreased during the next eighteen hours. There was an over-all retention of about 200 mEq. of sodium during the twenty-four hours following ACTH administration as compared with the control period. On the other hand, the rate of urinary excretion of potassium during the first six hours was slightly elevated above the pre-injection

closely related chemically to the cortical steroids than are the 17-ketosteroids. Although there is no direct evidence to support it, the belief is generally held that the adrenal cortex itself elaborates ketosteroids having anabolic and androgenic actions (34). However, it is possible that cortical steroids undergo degradation to 17-ketosteroids in the liver or other tissues. The lag in 17-ketosteroid excretion observed in our experiments could be explained by the latter possibility.

DISCUSSION

The doses of porcine ACTH used in these experiments are greater than those required to produce maximal stimulation of the adrenal cortex. Fifty mg. injected into M.J. produced as great a response as 100 mg. in M.L. as measured by the various metabolic alterations described above. Forsham *et al.* (2) considered 25 mg. given intramuscularly as providing a near-maximum response. The large quantities were employed in order to obtain ACTH levels in body fluids which could be readily detected by the assay method utilized and which might be expected to result in appreciable urinary excretion of the tropin. Despite the fact that high plasma titers of porcine ACTH were attained in human subjects, the hormone was not found in the urine in appreciable quantities. However, this does not necessarily mean that the ACTH elaborated by the human pituitary is not excreted in the urine.

The manifold changes that followed the intravenous infusion of ACTH were of short duration. The concentration of tropin in the plasma fell to control levels in less than three hours; the urinary excretion of steroids indicates that the secretory activity of the adrenal cortex had returned to normal within six hours; the metabolic changes in most instances reached their peak before the sixth hour; and practically all the constituents of the body fluids returned to control levels by the twelfth hour. It is therefore evident that a single injection of ACTH acts promptly upon the adrenal cortex and that the effects of this action persist for only a few hours.

The results of these experiments with ACTH in human subjects lend considerable support to a unitarian concept of pituitary tropic action on the adrenal cortex. ACTH produces changes in carbohydrate and protein metabolism which are to be expected of a substance which stimulates the adrenal cortex to secrete those steroids with an oxygen on C-11 and/or C-17. Furthermore, ACTH alters electrolyte metabolism in a manner which suggests that, under its influence, compounds similar to desoxycorticosterone in metabolic activity are being secreted from the adrenal cortex. Both 17-ketosteroids and steroids with a ketol side-chain are excreted in increased quantities following the administration of ACTH. These data indicate that pituitary tropins other than ACTH are not required to explain the manifold actions of the adrenal cortex, as has been

These results confirm the observations of Mason *et al.* (3) and Forsham *et al.* (2) and extend the study of urinary excretion of steroids to an examination of the effect of a single injection of ACTH. The results obtained in subject M.J. indicate not only that the adrenocorticotrophic hormone has a prompt stimulatory effect on the secretory activity of the adrenal cortex but also that the enhanced secretory activity of this gland soon ceases

TABLE 8. STEROIDS IN THE BODY FLUIDS

Subject	Urine collection periods*	Urinary neutral lipid reducing steroids, mg.† per hour	Urinary 17-ketosteroids, mg.‡ per hour
M.L.	Control day 1		0.43
	Control day 2		0.51
	0-24	0.28	0.72
	Control day 3	0.11	0.43
	Control day 4	0.11	0.39
M.J.	Control day 1	0.12	0.85
	Control day 2	0.17	0.81
	Control day 3	0.10	0.62
	Control day 4	0.12	0.62
	0-3	0.50	1.49
	3-6	0.44	1.76
	6-12§	0.19	0.58
	12-24	0.16	0.59
	Control day 5		0.58

* The numbers in this column indicate hours following the beginning of ACTH infusion.

† In terms of desoxycorticosterone acetate.

‡ In terms of androsterone.

§ Collection may have been incomplete. On basis of creatinine output, estimated to be 35 per cent low.

when the circulating level of ACTH again becomes normal. These findings are in agreement with the results of experimental work in animals which has demonstrated that a single dose of ACTH causes a very prompt depletion of adrenal cholesterol and ascorbic acid and that these constituents are restored to normal within a few hours (33).

The rapid drop in the rate of excretion of steroids after the maximal excretory level is reached suggests that these substances, when acutely mobilized, have but a brief sojourn in the body. The peak rate of excretion of urinary 17-ketosteroids appears to occur later than that of the neutral-lipid reducing steroids. The neutral-lipid reducing substances are more

closely related chemically to the cortical steroids than are the 17-ketosteroids. Although there is no direct evidence to support it, the belief is generally held that the adrenal cortex itself elaborates ketosteroids having anabolic and androgenic actions (34). However, it is possible that cortical steroids undergo degradation to 17-ketosteroids in the liver or other tissues. The lag in 17-ketosteroid excretion observed in our experiments could be explained by the latter possibility.

DISCUSSION

The doses of porcine ACTH used in these experiments are greater than those required to produce maximal stimulation of the adrenal cortex. Fifty mg. injected into M.J. produced as great a response as 100 mg. in M.L. as measured by the various metabolic alterations described above. Forsham *et al.* (2) considered 25 mg. given intramuscularly as providing a near-maximum response. The large quantities were employed in order to obtain ACTH levels in body fluids which could be readily detected by the assay method utilized and which might be expected to result in appreciable urinary excretion of the tropin. Despite the fact that high plasma titers of porcine ACTH were attained in human subjects, the hormone was not found in the urine in appreciable quantities. However, this does not necessarily mean that the ACTH elaborated by the human pituitary is not excreted in the urine.

The manifold changes that followed the intravenous infusion of ACTH were of short duration. The concentration of tropin in the plasma fell to control levels in less than three hours; the urinary excretion of steroids indicates that the secretory activity of the adrenal cortex had returned to normal within six hours; the metabolic changes in most instances reached their peak before the sixth hour; and practically all the constituents of the body fluids returned to control levels by the twelfth hour. It is therefore evident that a single injection of ACTH acts promptly upon the adrenal cortex and that the effects of this action persist for only a few hours.

The results of these experiments with ACTH in human subjects lend considerable support to a unitarian concept of pituitary tropic action on the adrenal cortex. ACTH produces changes in carbohydrate and protein metabolism which are to be expected of a substance which stimulates the adrenal cortex to secrete those steroids with an oxygen on C-11 and/or C-17. Furthermore, ACTH alters electrolyte metabolism in a manner which suggests that, under its influence, compounds similar to desoxycorticosterone in metabolic activity are being secreted from the adrenal cortex. Both 17-ketosteroids and steroids with a ketol side-chain are excreted in increased quantities following the administration of ACTH. These data indicate that pituitary tropins other than ACTH are not required to explain the manifold actions of the adrenal cortex, as has been

suggested by Selye (35) and Albright (34). These investigators have suggested that the elevated androgen titers which appear to be present in body fluids when the adrenal cortex is stimulated to hyperactivity are due to the adrenal cortical secretion of androgen in response to a pituitary tropin other than ACTH. However, it is just as reasonable to suppose that the androgenic steroids result from the incomplete metabolism of cortical steroids which are secreted in abnormally high concentrations in response to excessive stimulation by ACTH itself.

It is important to know whether tissues other than the adrenal cortex respond directly to ACTH. If this is true, then certain of the metabolic changes described in this paper were not necessarily mediated via the adrenals. An almost complete answer to this question has been supplied by the experiments of Forsham *et al.* (2) who have demonstrated that a patient with Addison's disease treated with ACTH for four days failed to show the carbohydrate, electrolyte, hematologic and urinary steroid alterations which occurred when individuals with functional adrenal cortical tissue were similarly treated. It remains to be proven whether the serum peptidase changes herein reported are likewise dependent upon functional adrenal cortical tissue.

In view of the fact that large doses of ACTH were employed, the question may legitimately be asked whether the metabolic and other changes described in this report have any relevance for normal pituitary-adrenal function. It is possible that comparably high titers of ACTH never exist in human body fluids even under conditions of severe stress, when maximal discharge of ACTH can be anticipated. Although we are not prepared to deny that such large doses of ACTH may stimulate the adrenal cortex in a manner which is not physiologic, we believe the possibility is remote. Nevertheless, this point deserves further study. The following considerations suggest that the ACTH doses employed, even though maximal, are not inordinately excessive. The normal adult pituitary contains an amount of ACTH equivalent in activity to that of 20 mg. of the standard preparation employed by us (unpublished observations). The results of experiments in the rat indicate that a severe acute stress causes the release of approximately 50 per cent of the ACTH stored in the rat pituitary. If the same conditions obtain in man, then it is possible for 10 mg. (in terms of the standard) of ACTH to be released into the blood stream of a human subject within a very short period of time. The doses used in our experiments were only five- to tenfold greater than this amount and were infused over a period of one hour.

SUMMARY

1. Large single doses of pituitary ACTH were administered to 2 healthy young adults by slow intravenous infusion.

2. No change in blood pressure occurred in either subject. The only untoward reaction was a mild chill and fever experienced by one of the subjects.

3. The tropin disappeared rapidly from the plasma, reaching control levels two hours after the infusion was terminated. Negligible quantities of ACTH appeared in the urine.

4. Fasting blood sugar was not influenced by ACTH. However, glycosuria and a diabetic type of blood sugar response followed the ingestion of a meal six hours after the start of the infusion.

5. Urinary nitrogen and uric acid were elevated.

6. A marked decrease in the number of circulating lymphocytes and eosinophils occurred concomitantly with an increase in the number of circulating neutrophils.

7. The electrophoretic distribution of serum proteins was unchanged.

8. The titers of antistreptolysin O, isoagglutinins, typhoid H and O and diphtheria antibodies were uninfluenced.

9. Certain serum peptidases underwent small but inconsistent increases.

10. There was an elevation in the pH of the blood of one subject which appeared to be related to the chill and fever. There was no change in either the pH or CO₂ of the plasma of the other subject.

11. Plasma sodium and chloride concentrations remained unchanged, but plasma potassium declined significantly in both subjects at a time when the other metabolic effects were maximal.

12. There was an early increase in the urinary excretion of potassium followed by return to a normal or slightly subnormal potassium excretion from the sixth to the twenty-fourth hour. One subject showed a slight retention of sodium and chloride during the first six hours while the other subject, in whom an inhibition of a water diuresis occurred, showed sodiumphoresis and chloruresis during this same period. There was a marked retention of sodium and chloride in both subjects from the sixth to the twenty-fourth hour.

13. The quantity of 17-ketosteroids and of neutral-lipid reducing substances in the urine was markedly elevated. The peak rate of excretion of these substances occurred at about three hours after ACTH administration and their level in the urine returned to normal by the twelfth hour.

14. The sequence of metabolic and other events was such that the secretory activity of the adrenal cortex appeared to reach a maximum by the third hour after ACTH administration and to return to pretreatment activity by the sixth hour. Most of the alterations in metabolic constituents reached a maximal deviation from pretreatment levels at the sixth hour; the values returned to normal by the twelfth hour.

15. The significance of the observed results for pituitary-adrenal function is discussed.

suggested by Selye (35) and Albright (34). These investigators have suggested that the elevated androgen titers which appear to be present in body fluids when the adrenal cortex is stimulated to hyperactivity are due to the adrenal cortical secretion of androgen in response to a pituitary tropin other than ACTH. However, it is just as reasonable to suppose that the androgenic steroids result from the incomplete metabolism of cortical steroids which are secreted in abnormally high concentrations in response to excessive stimulation by ACTH itself.

It is important to know whether tissues other than the adrenal cortex respond directly to ACTH. If this is true, then certain of the metabolic changes described in this paper were not necessarily mediated via the adrenals. An almost complete answer to this question has been supplied by the experiments of Forsham *et al.* (2) who have demonstrated that a patient with Addison's disease treated with ACTH for four days failed to show the carbohydrate, electrolyte, hematologic and urinary steroid alterations which occurred when individuals with functional adrenal cortical tissue were similarly treated. It remains to be proven whether the serum peptidase changes herein reported are likewise dependent upon functional adrenal cortical tissue.

In view of the fact that large doses of ACTH were employed, the question may legitimately be asked whether the metabolic and other changes described in this report have any relevance for normal pituitary-adrenal function. It is possible that comparably high titers of ACTH never exist in human body fluids even under conditions of severe stress, when maximal discharge of ACTH can be anticipated. Although we are not prepared to deny that such large doses of ACTH may stimulate the adrenal cortex in a manner which is not physiologic, we believe the possibility is remote. Nevertheless, this point deserves further study. The following considerations suggest that the ACTH doses employed, even though maximal, are not inordinately excessive. The normal adult pituitary contains an amount of ACTH equivalent in activity to that of 20 mg. of the standard preparation employed by us (unpublished observations). The results of experiments in the rat indicate that a severe acute stress causes the release of approximately 50 per cent of the ACTH stored in the rat pituitary. If the same conditions obtain in man, then it is possible for 10 mg. (in terms of the standard) of ACTH to be released into the blood stream of a human subject within a very short period of time. The doses used in our experiments were only five- to tenfold greater than this amount and were infused over a period of one hour.

SUMMARY

1. Large single doses of pituitary ACTH were administered to 2 healthy young adults by slow intravenous infusion.

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determinations. Creatinine was determined by the method of Bonsnes and Taussky (4), adapted to the Coleman jr. spectrophotometer. In no instance was there evidence of a significant error in the collection of urine. Serum representative of each twenty-four hour urine specimen was prepared by pooling serums obtained at the beginning and at the end of each collection period.

3. *Bioassay of chorionic gonadotropin.* The method of Albert (5) was used.

4. *Calculations.* Renal clearance was calculated by the conventional formula UV/B , where U is the concentration of the hormone in the urine, B is its concentration in the serum and V is the volume of urine excreted per minute. The serum-to-urine ratio was calculated by dividing urine concentration into serum concentration. Creatinine coefficients were calculated by dividing the milligrams of creatinine excreted in each twenty-four hour period by the weight of the subject in kilograms.

TABLE 1. CONCENTRATION OF CHORIONIC GONADOTROPIN IN URINE AND SERUM, AND RELATED VALUES

Subject number and diagnosis	Days past last menstrual period	Urine concentration, I.U. per ml.	Gonadotropin in urine, I.U. per 24 hours	Serum concentration, I.U. per ml.	Ratio of serum to urine concentrations	Clearance (UV/B), ml. per minute	Creatinine excretion, Gm. per 24 hours	Creatinine coefficient
Subject 1 Normal pregnancy	95	23.8	28,500	24.2	1.02	0.79	1.12	21.1
	105	17.8	30,300	58.7	3.30	0.36	1.02	19.2
	114	10.4	12,700	36.8	3.54	0.24	1.06	20.1
	127	8.1	11,700	29.5	3.66	0.27	0.99	18.7
	165	4.9	6,400	16.9	3.45	0.26	0.96	16.0
	213	12.1	15,100	21.1	1.74	0.50	1.03	16.9
	261	7.2	8,500	17.3	2.40	0.34	1.12	17.5
Subject 2 Normal pregnancy	80	118.5	205,000	344.0	2.90	0.43	1.43	21.4
	89	40.3	66,100	90.6	2.25	0.51	1.52	22.7
	101	25.5	47,900	105.3	4.13	0.32	1.73	25.9
	131	34.4	47,100	86.6	2.52	0.38	1.66	23.7
	163	10.3	15,200	20.8	2.01	0.51	1.34	18.7
Subject 3 Normal pregnancy	111	7.6	17,500	21.7	2.86	0.56	1.26	20.1
	118	4.1	6,500	16.1	3.93	0.34	1.44	22.5
	139	12.7	19,000	44.8	3.53	0.30	1.27	19.8
	204	10.8	15,500	35.4	3.28	0.33	1.32	19.5
	259	10.5	12,000	32.2	3.07	0.26	1.18	17.4
Subject 4 Normal pregnancy	70	105.0	172,000	401.0	3.82	0.30	0.98	21.4
	83	95.0	147,300	261.0	2.75	0.39	0.98	21.3
	118	12.3	25,800	61.4	5.00	0.29	0.90	18.9
Subject 5 Testicular tumor	—	10.0	10,000	—	—	—	2.27	29.8
	—	9.6	9,400	28.0	2.92	0.23	2.62	34.5
Subject 6 Hydatidiform mole	—	11.6	4,600	—	—	—	0.94	18.8
	—	18.3	5,500	17.2	0.94	0.22	0.92	18.5
	—	7.0	7,100	11.8	1.69	0.42	1.04	20.8

THE RENAL CLEARANCE OF CHORIONIC GONADOTROPIC HORMONE IN PREGNANCY, IN NEOPLASM OF THE TESTIS AND IN HYDATIDIFORM MOLE*

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AS IS well known, chorionic gonadotropin appears in the urine of pregnant women twenty-five to forty-five days after the first day of the last menstrual period. The rate of excretion reaches a peak at fifty to sixty days after the last menstrual period and then falls to a rather constant level, which is maintained until the hormone disappears from the urine several days after delivery. These rather remarkable variations in the excretion of chorionic gonadotropin were first clearly described by Browne and Venning (1) in 1936, and similar changes in the serum concentration of this hormone were reported by Boycott and Rowlands (2) in 1938. These authors and others have speculated whether such variations are the result of change in the rate of formation, in the rate of destruction or in the rate of excretion of the hormone. The observations that we wish to report in the present paper were obtained in order to define more precisely the role of the kidneys in the excretion of chorionic gonadotropin by determining the renal clearance of this hormone in various clinical states.

METHODS

1. *Subjects.* The patients studied consisted of 4 normal pregnant women, a man with testicular chorioma and a woman with hydatidiform mole, in none of whom was there clinical or laboratory evidence of renal disease at any time. The patients are briefly described in the appendix.¹

2. *Collection of Specimens.* The accuracy of the twenty-four hour collection of urine required for each clearance was confirmed by creatinine

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¹ One pregnant subject, included in our abstract (3), has been excluded from the present report because signs of toxemia subsequently developed.

serum concentration was 28 i.u. per milliliter and the urine concentration was 9.6 i.u. per milliliter. Thus the concentration of the hormone in the tumor was some eight times as great as in the serum.

3. *Hydatidiform mole*. The subject was excreting moderate amounts of chorionic gonadotropin derived from the degenerating remnant of a hydatidiform mole which was imbedded in the wall of her uterus. Clearances (Table 1) were 0.22 and 0.42 ml. per minute preoperatively and on the first day after hysterectomy the clearance was 0.32 ml. per minute. The hor-

TABLE 3. RENAL CLEARANCES OF CHORIONIC GONADOTROPIN IN NORMAL PREGNANCY

	Observations	Clearances, milliliters per minute			Serum Urine ratios		
		Mean	S.D.*	Range	Mean	S.D.*	Range
First trimester, 0-92 days†	4	0.41 ± 0.04‡	0.09	0.30-0.51	2.93 ± 0.33‡	0.65	2.25-3.82
Second trimester, 93-185 days	12	0.38 ± 0.05	0.16	0.24-0.79	3.24 ± 0.32	1.10	1.02-5.00
Third trimester, 186-280 days	4	0.36 ± 0.05	0.10	0.26-0.50	2.62 ± 0.35	0.70	1.74-3.28
Entire pregnancy, 0-280 days	20	0.38 ± 0.03	0.13	0.24-0.79	3.06 ± 0.19	0.84	1.02-5.00

* Standard deviation.

† Days after the first day of the last menstrual period.

‡ Standard error of the mean.

mone quickly disappeared from the urine, and by the second day after operation gonadotropic activity could no longer be measured.

COMMENT

The two devices of renal clearance and ratios of serum-to-urine concentration were selected as the best means for portraying the activity of the kidneys in excreting chorionic gonadotropin. The concept of renal clearance is a familiar one and has been used in the study of the excretion of a large number of substances. Although no one to our knowledge has previously used renal clearances as a means of studying the excretion of hormones, data are available from which such calculations can be made. From

RESULTS

1. *Normal pregnancy.* Table 1 illustrates the values obtained in the 4 normal subjects for each of twenty determinations. In agreement with the work of Browne and Venning and of Boycott and Rowlands, high levels of the hormone in urine and serum were observed in the first trimester and relatively small amounts were present in the latter two-thirds of pregnancy. This table shows further that creatinine was excreted at a rate rather constant for each subject and that in no instance was there evidence of significant error in the collection of urine. The creatinine coefficient on the other hand tended to diminish with advancing pregnancy as body weight increased.

Table 2 shows the average clearance obtained for each subject. The clearances did not vary significantly among the 4 subjects. The average ratio of serum concentration to urine concentration of the hormone for each subject also exhibited relatively little variation between subjects.

TABLE 2. RENAL CLEARANCES OF CHORIONIC GONADOTROPIN IN NORMAL PREGNANCY

Subject	Observations	Clearances, milliliters per minute			Serum Urine ratios		
		Mean	S.D.*	Range	Mean	S.D.*	Range
1	7	0.39 ± 0.07 †	0.20	0.24-0.79	2.73 ± 0.39 †	1.32	1.02-3.66
2	5	0.43 ± 0.04	0.08	0.32-0.51	2.76 ± 0.37	0.84	2.01-4.13
3	5	0.36 ± 0.05	0.12	0.26-0.56	3.33 ± 0.19	0.42	2.86-3.93
4	3	0.33 ± 0.03	0.05	0.29-0.39	3.86 ± 0.65	1.13	2.75-5.00

* Standard deviation.

† Standard error of the mean.

Table 3 gives the mean clearances and ratios for all 4 normal subjects according to trimester. The average clearance for the first trimester was 0.41, for the second, 0.38, and for the third, 0.36 ml. per minute. The minimal clearance observed was 0.24, the maximum was 0.79, and the average for all twenty determinations in normal pregnancy was 0.38 ml. per minute. The concentration of chorionic gonadotropin in the serum was found to average 3.06 times its concentration in the urine.

2. *Testicular tumor.* A single clearance was determined on a man with a mixed adenocarcinoma and chorioma, grade 4 (Broders' method). The clearance was 0.23 ml. per minute, and the serum-to-urine ratio was 2.92, values consistent with those observed in normal pregnancy. A saline extract of the tumor assayed 220 I.U. per gram of tissue (wet), while the

APPENDIX

Synopsis of Cases

Case 1. The patient was a white primipara, primigravida, aged 21 years, who had no history of renal disease. Blood pressure and results of urinalysis remained within normal limits throughout the course of her pregnancy, which was terminated by spontaneous delivery of a normal healthy infant.

Case 2. The patient was a white para II, gravida III, aged 20 years, who had pyuria after a spontaneous miscarriage two years before the present study. She had had a normal pregnancy terminated one year previously by spontaneous delivery of a healthy infant. During the pregnancy studied there was no evidence of renal disease or of hypertension.

Case 3. The patient was a white para II, gravida II, aged 27 years, who had had an uneventful pregnancy two years before the present study. Blood pressure and results of urinalysis remained within normal limits throughout the course of the present pregnancy, which has resulted in a healthy infant.

Case 4. The patient was a white primipara, primigravida, aged 24 years, who had had no renal disease. No abnormality of the present pregnancy has been observed to the time of this writing (August, 1948).

Case 5. The patient was a white man, aged 27 years, who had noted a nontender firm enlargement of his right testicle in December, 1947. He was seen six weeks later when the mass was estimated to be 6.5 by 5 by 5 cm. A small disk of breast tissue was palpable beneath each nipple. Orchiectomy was done February 10, 1948, and a spherical nodule 3 cm. in diameter was found in this testicle. The histologic diagnosis was "mixed grade 4 adenocarcinoma and chorionepithelioma." Laboratory data of significance are as follows: 17-ketosteroids, 13.1 mg. per 24 hours; cortin-like substances, 0.76 mg. per 24 hours; estrogens, 48 rat units per 24 hours before orchiectomy and 21 rat units per 24 hours after removal of the tumor.

Case 6. The patient was a para II, gravida IV, aged 36 years, who had expelled a hydatidiform mole three months before coming to the Mayo Clinic. Dilatation and curettage had been done twice, but the Friedman reaction remained positive. Total abdominal hysterectomy was done, and a small nodule of degenerating placental tissue was found embedded in the wall of the uterine fundus.

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the work of Smith and Smith (6, 7) an average clearance of 0.85 ml. per minute is obtained from a total of thirty-nine of their determinations on normal pregnant subjects with a rather wide range of variation. The data of Taylor and Scadron (8) permit similar computations. The average clearance for twenty-three determinations on 17 normal subjects was 0.44 ml. per minute. In the present investigation, a mean clearance of 0.38 ml. per minute was obtained for normal pregnant women, a value agreeing very well with those derived from the data of Taylor and Scadron.

The ratio of the concentration of certain substances in the serum to their concentration in the urine has been shown to be a highly significant quantity by the studies of von Rhorer (9), Addis (10) and Newburgh (11). Although it is very doubtful that such a ratio for chorionic gonadotropin has the significance that it would have for urea or sodium, the concept is a useful one and therefore is used in the presentation of this material. Although the data of Smith and Smith and of Taylor and Scadron are not adequate to permit the calculation of relative concentrations of chorionic gonadotropin in the urine and in the serum, the assumption of a urinary excretion rate of 1 to 2 ml. per minute would mean that the serum concentration was found to be from 0.86 to 4.54 times that of the urine. In the present study the serum concentration was found to be from 1.02 to 5.00 times as great as the urine concentration, with an average ratio of 3.06.

It is interesting that the clearances and ratios obtained in the instances of the man who had the testicular tumor and of the woman who had the hydatidiform mole were essentially the same as for the normal pregnant woman. This would suggest that the kidneys in nonpregnant women or in men excrete chorionic gonadotropin in the same manner as do the kidneys in pregnancy.

Since the clearance of chorionic gonadotropin was much the same in all cases, it appears that variations in serum and urine concentrations must depend on changes in the rate of formation or in the rate of destruction of the hormone. Studies are in progress to evaluate the relative importance of these factors.

SUMMARY

The renal clearance of chorionic gonadotropin has been determined during pregnancy, in hydatidiform mole, and in testicular chorioma. The mean clearance in pregnancy was 0.38 ml. per minute, and closely similar values were observed in hydatidiform mole and in testicular chorioma. The constancy of values obtained in the three trimesters of pregnancy indicates that the remarkable fluctuation of chorionic gonadotropin in pregnancy is due to changes in rates of destruction or of formation and not to differences in renal excretion.

COARCTATION OF THE AORTA ASSOCIATED WITH ABNORMAL DIGITS, OVARIAN INSUFFICIENCY AND SHORTNESS OF STATURE*

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IN describing a syndrome characterized by primary ovarian insufficiency and decreased stature, Albright, Smith and Fraser (1) noted the presence of coarctation of the aorta in 1 patient of 11. In 5 others the diastolic blood pressure was 90 mm. of mercury or more. Pich (2), Ornstein (3) and Wilkins and Fleischmann (4) each recorded a case exhibiting ovarian insufficiency, decreased stature and coarctation. Lisser and his associates (5), in a group of 25 patients with congenitally aplastic ovaries and other associated abnormalities, described coarctation of the aorta in 1 and an anomalous toe in another.

We have recently studied 9 males and 5 females affected with coarctation of the aorta (6). We were impressed with finding that 4 of the females manifested shortness of stature and abnormal digits, and that the 3 who were adults showed clinical evidence of ovarian hypofunction. Two of these exhibited diastolic blood pressures over 90 mm. of mercury.

These observations bear on any general consideration of the nature of the syndrome of so-called ovarian agenesis. They also lead one to the supposition that those affected with this syndrome should be studied carefully for the presence of coarctation of the aorta and its often serious cardiovascular complications. Hence, we are reporting the 4 patients briefly.

CASE REPORTS

Case 1, J. F., was a 24-year-old single white woman of limited intelligence who gave a history of abnormally short stature since childhood. At the age of 13 she had not menstruated and because of this, she consulted a physician who induced one menstrual period after a series of injections of unknown nature. Breast tissue had not been palpable until stilbestrol therapy had been employed for several months. Vaginal bleeding occurred at irregular intervals while the patient received stilbestrol but had ceased upon withdrawal of the drug. The patient was 56 inches in height and weighed 110 pounds. The blood pressure in the right arm was 170 mm.Hg systolic and 112 diastolic. It was not

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Case 2, O. P., was a 36-year-old colored woman who said she had menstruated once at the age of 13 years. However, her intelligence was limited and the reliability of her history is poor. Breast tissue was not present and the genitalia were infantile. Axillary and pubic hair was sparse. After therapy with stilbestrol, however, breast tissue became evident and pubic and axillary hair more abundant. Vaginal bleeding occurred at irregular intervals while the patient received this drug. She was 56 inches in height with an arm span of 65 inches and weighed 108 pounds. Her neck was short (Fig. 2). Upon examina-

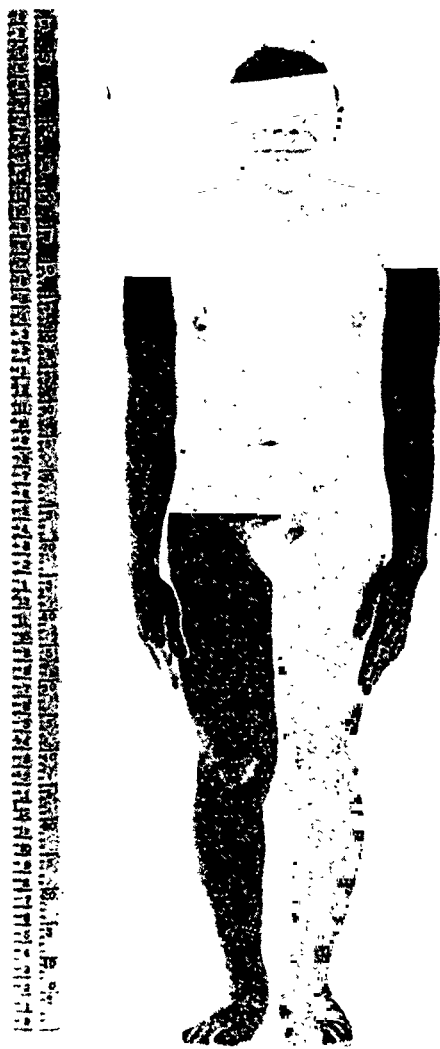


FIG. 2. Photograph of the patient in Case 2 showing the short stature, short neck, and the relatively long upper extremities. The pubic hair and breast development were the result of therapy with stilbestrol.

tion of the extremities, the fourth toe of the left foot was found to be abnormally short (Fig. 3). In x-ray photographs this abnormality was found to be due to a shortening of the metatarsal and phalangeal bones. Intravenous pyelograms showed normal kidneys. Because abnormal digits, shortness of stature, and hypo-ovarianism had been seen in the other cases of coarctation of the aorta here reported, special examinations for its presence were made. The diagnosis of subclinical coarctation was established by direct measurements of arterial pressure in the upper and lower extremities and by retrograde aortic

obtainable in the legs. Axillary and pubic hair was sparse. The genitalia were infantile and the ovaries could not be palpated. No vaginal smear was made. The fourth finger of the right hand and the fourth toes of both feet were abnormally short. The tibiae showed slight bowing. The basal metabolic rate was plus 8 per cent. X-ray examination revealed the fourth right metacarpal and fourth metatarsal and phalangeal bones of both feet to be abnormally short (Fig. 1). The diagnosis of coarctation of the aorta was made on the basis of differences in blood pressure between the upper and lower extremities, the presence of collateral circulation, and notching of the ribs shown by x-ray examination.

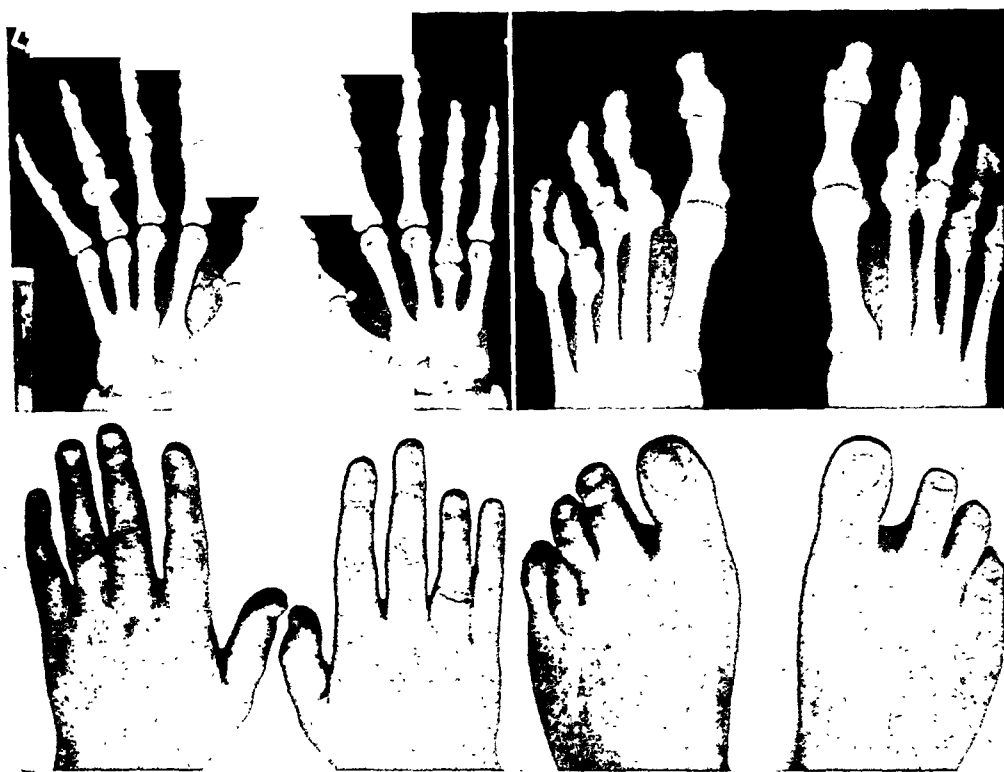


FIG. 1. Roentgenograms and photographs of the hands and feet of the patient in Case 1. There is a decrease in the length of the fourth right metacarpal bone. The shaft is narrow, the base decreased in width and the head deformed. The fourth right metatarsal is decreased somewhat in length but the phalanges are of normal size. The fourth left metatarsal and the proximal phalanx are considerably shortened.

Operative repair of the coarctation was performed by Dr. Thomas H. Burford. Menstrual function, twenty-four months later, had not returned. The blood pressure by direct puncture, eleven months postoperatively was 113 mm.Hg systolic and 75 diastolic in the right brachial artery and 132 systolic and 78 diastolic in the right femoral artery. Twenty-one months after operation, the blood pressure in the right brachial artery was 130 systolic and 75 diastolic while that in the right femoral artery was 132 systolic and 78 diastolic. The blood pressures by the auscultatory method were 140 systolic and 80 diastolic in the arm and 150 systolic and 84 diastolic in the legs.

in height with an arm span of 54 inches and she weighed 95 pounds. Axillary and pubic hair was scanty, and the genitalia were infantile. Breast tissue was not palpable. Stilbestrol therapy caused the breasts to develop, axillary and pubic hair to become more dense, and vaginal bleeding to occur at intervals. The blood pressure in the right brachial artery determined by direct puncture was 143 mm.Hg systolic and 60 diastolic; that in the right femoral artery was 90 systolic and 57 diastolic. The basal metabolic rate was minus 11 per cent. Examination of the extremities revealed that the fifth left finger was short-



FIG. 4. Retrograde arteriogram in Case 2, taken 3 seconds after the injection of 70 per cent diodrast into the left common carotid artery. A slight degree of constriction of the descending aorta is indicated by the arrow at the usual locality of coarctation. No collateral circulation was seen.

ened. X-ray photographs showed that the distal phalanx of this finger was rudimentary. In addition, there was failure of closure of the epiphyseal joints of the radius and ulna. Intravenous pyelograms revealed caliectasis of the left kidney. X-ray examination of the chest revealed an azygos lobe of the right lung. Diagnosis of coarctation was made on the basis of diminished blood pressure in the lower extremities as compared to the

arteriography (7). The femoral systolic pressure was 162 mm.Hg and that in the brachial artery, 193; diastolic pressure was 107 mm.Hg in the femoral artery and 116 in the brachial. Usually the femoral systolic pressure is about 20 mm.Hg higher than the brachial. The coarctation was of only mild degree (Fig. 4). In addition, the patient suffered from generalized arterial hypertension. She had a slipped epiphysis of the right femur with cystic changes in the femoral head and right acetabulum. The basal metabolic rate was plus 7 per cent. This case was similar in many respects to one reported by Stewart and Bailey, *cf.* their case number 11 (8).

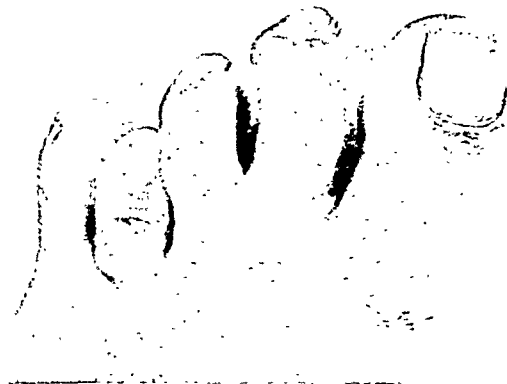


FIG. 3. X-ray photograph (oblique) of the left foot of the patient in Case 2. The fourth left metatarsal and all of its phalanges are shortened. In addition, this patient showed ununited epiphyses of the iliac crest, indicating a bone age of approximately 18 years. The appearance of the toe is shown on the right.

Case 3, N. P., was an 8-year-old white girl who complained of dyspnea of two months' duration. She had suffered three attacks of rheumatic fever with damage to her aortic and mitral valves. She was 39.5 inches in height and weighed 64 pounds. The minimum average height for an 8-year-old girl is 43 inches (9). The blood pressure in the right brachial artery by direct arterial puncture was 214 mm.Hg systolic and 57 diastolic; in the left brachial artery 124 systolic and 57 diastolic; and in the right femoral artery 87 systolic and 54 diastolic. Examination of the feet revealed short third toes which, on x-ray examination, proved to be caused by shortening of the proximal phalanges. The diagnosis of coarctation of the aorta was established by differences in blood pressure levels between the upper and lower extremities, the presence of collateral circulation, notching of the ribs in x-ray photographs, by retrograde aortic arteriography, and by exploratory thoracotomy. The subclavian artery originated at the site of the coarctation. She died at operation.

Case 4, F. R., was a 22-year-old white single woman whose chief complaints were dyspnea, fatigability and hypertension of twenty-one months' duration. A lateral nystagmus of the eyes had been present since birth. She was of limited intelligence, having been graduated from high school at age 21. She had never menstruated. She was 55 inches

It is possible that the occurrence of coarctation in primary hypo-ovarianism is more frequent than previously reported, particularly in view of the common finding of slight to moderate elevation of the blood pressure in this condition (4). An alternative cause of the hypertension occurring in some patients with primary hypo-ovarianism may lie in congenital anomalies of the kidneys observed by Plachte (14) in patients with syndromes thought to be related to that of ovarian agenesis. Possibly by more specialized methods of study, such as direct measurements of arterial pressure and retrograde aortic arteriography, the milder form of coarctation might be demonstrated when these other congenital abnormalities exist.

SUMMARY

1. The occurrence of abnormal digits and shortness of stature in 4 females with coarctation of the aorta has been described. Three of the patients exhibited signs of ovarian insufficiency; the fourth patient had not reached puberty. The degree of coarctation was minimal in 1 case, requiring specialized techniques for making the diagnosis.

2. A review of the literature suggests that a slight to moderate degree of hypertension not infrequently accompanies the syndrome of primary ovarian insufficiency and decreased stature. Coarctation was reported to be demonstrable in only a few.

3. It is suggested that coarctation of the aorta should be suspected in women with primary ovarian hypofunction, abnormal digits, and an elevated blood pressure.

Acknowledgments

We are indebted to Drs. Thomas H. Burford and Wendell G. Scott for the permission to reproduce Figure 4; to Dr. Merl J. Carson for referring Case 3; and to Dr. Otto H. Grunow for the radiologic interpretations. The x-ray photographs were made by the Department of Radiology and the reproductions and other photographs by the Department of Illustration.

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upper, the presence of collateral circulation, notching of the ribs in x-ray photographs, and retrograde aortic arteriography. A surgical repair of the coarctation was performed by Dr. Burford in January, 1949, with excellent immediate results.

COMMENTS

The 4 females discussed in this report exhibited several of the characteristics of a general syndrome described by Turner (10), Albright and his associates (1) and others (2, 3, 4). Although determinations of urinary follicle-stimulating hormone and 17-ketosteroids were not done, presumptive evidence for hypo-ovarianism was present on the basis of developmental and menstrual history, physical findings, the scantiness of the pubic and axillary hair, and the growth of this hair following therapy with estrogens. It is probable, therefore, that these were not cases of pituitary dwarfism.

Although 4 of the 5 females in our group of 14 patients with coarctation of the aorta (6) manifested associated skeletal and sexual abnormalities, these phenomena, with the exception of shortness of stature in 3, were not observed in any of the 9 males. Webbing of the neck was not present in any case. The shortened digits were bilateral in 2 of the cases, no more than a single abnormal digit being present in any one hand or foot. In 2 of the patients, the metacarpal or metatarsal bones were primarily at fault and, in two others, the phalanges.¹ Generalized osteoporosis was not observed.

Ovarian agenesis is associated with a multiplicity of congenital defects. The cases reported here exhibited some of the anomalies noticed by others (4). Mental retardation, while not tested for, was obviously present. An eye abnormality, short neck, cystic bony changes, retardation of growth of bone, azygos lobe of the lung, and anomaly of the renal pelvis were each found. The shortening of the digits did not appear to be similar to those observed by Albright and associates (12) in pseudohypoparathyroidism (Seabright-Bantam syndrome) since in our cases only one digit of an extremity was involved. The x-ray appearance, however, resembled that seen in a case of J. S. L. Browne shown by Selye (13).

It is of interest that in Case 2 coarctation of the aorta was suspected on the basis of previous experience because of the presence of ovarian hypofunction, short stature, and abnormal digits in a patient with hypertension. Direct measurements by a Hamilton manometer of the blood pressure in the brachial and femoral arteries suggested confirmation of the diagnosis. Retrograde arteriography revealed a slight but definite degree of coarctation (Fig. 4).

¹ Albright has noted abnormal digits in 2 cases of ovarian insufficiency (11).

OVARIAN GRANULOSA CELL TUMOR AND ACROMEGALY

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AMENORRHEA is one of the most consistent and usually one of the earliest symptoms of pituitary tumor in women. Menstrual function may return, however, even after a prolonged period of amenorrhea, following surgical removal of the tumor, its shrinkage by roentgen irradiation, or possibly in rare instances following the escape of the expanding tumor from the sella turcica with consequent decompression of the normal elements of the gland. In the following unique case resumption of uterine bleeding occurred in an acromegalic as the result of a coincidental lesion, namely, a granulosa cell tumor of the ovary.

Case report.—The patient was a 42-year-old white, married, Russian-born housewife, who was first seen at the Columbia-Presbyterian Medical Center September 16, 1948, with the complaint of enlargement of the hands and feet and distortion of the face. These symptoms, first observed by the patient about six years before, had been preceded for about three years by excessive fatigue and frequent blurring of vision, beginning soon after the birth of her only child in 1939. More recently headache, weakness of the arms, tremors, a feeling of fullness in the mouth, and spreading of the teeth were noticed.

Her menstrual periods, which began at the age of 11½ years, had always been normal until 1945, when the patient had several profuse flows with some intermenstrual bleeding. A curettage performed at another hospital disclosed mild endometrial hyperplasia. The menses resumed their normal regularity until June 1946, when they ceased. From October 1944 to June 1948 the patient was treated intermittently with injections of estrogenic hormone because of hot flashes, fatigue, and depression, but these symptoms were much diminished for the five or six months before admission.

In January 1948 vaginal spotting occurred and the patient's physician removed a cervical polyp. In June 1948 scanty bleeding occurred for three days. Similar flows recurred in July, twice in August, and three times in September. Profuse bleeding began October 16 and continued for nine days, at the end of which time the patient was hospitalized.

Her appearance was typical of acromegaly (Fig. 1), with increase in size of the hands, feet, and facial features. Speech was thick. The optic discs showed bilateral temporal pallor but perimetric examination was unreliable because of poor cooperation. On pelvic examination a firm, freely movable, globular mass about 4 cm. in diameter was felt in the left adnexal region. The patient's weight was 61.1 Kg. and her basal metabolic rate, plus 14 per cent. Urinary estrogen excretion, determined by the fluorometric method of Jailer (1), was 32 micrograms in 24 hours. This value is higher than is normally encountered in postmenopausal women. All other chemical and serologic tests of blood, urine, and cerebrospinal fluid gave normal values. Stereoscopic roentgenologic examina-

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tion of the skull revealed a markedly enlarged sella turcica measuring 22×16 mm., with depression of its floor and thinning of the posterior clinoids (Fig. 2).

In addition to her obvious acromegaly the patient was believed to have a solid tumor of the left ovary, possibly of the granulosa cell type. On November 19, 1948, complete abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The uterus measured $9.5 \times 4.5 \times 3$ cm. and was normal save for a slender endometrial polyp 2.8 cm. long (Fig. 3), and adenomatous hyperplasia in a thin endometrium which showed evidence of previous atrophy (Fig. 4). There was moderate squamous metaplasia in the endocervix. The right ovary was grossly normal. It contained a simple cyst about 1 cm. in diameter, several graafian follicles, and a rather dense stroma with no evidence of recent ovulation. The left ovary was replaced by a solid, encapsulated tumor measuring 3.5 cm. in diameter. It had a white, whorled appearance when cut. Microscopic sections

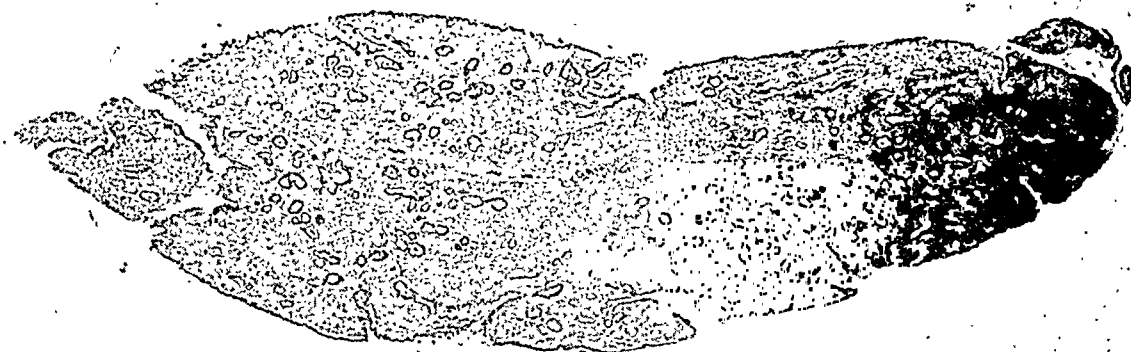


FIG. 3. Endometrial polyp.

showed a well preserved typical granulosa cell tumor. The cells were arranged in a cylindromatous pattern, with the cords of tumor cells separated by a rather dense stroma and presenting the characteristic appearance of moiré silk (Fig. 5).

Postoperative convalescence was uneventful and the patient was discharged on December 2, 1948, to continue radiotherapy to the pituitary region which was begun prior to operation.

COMMENT

Despite the high incidence of amenorrhea in women with acromegaly, there is a dearth of information concerning the genital organs of these patients (2, 3). Cushing and Davidoff (4), in their detailed treatise on the pathologic findings in acromegaly, were able to find in the literature only 4 cases in which the female pelvic organs were adequately described. More recently Henderson (5) and Klöppner (6) have written excellent papers on the sexual dysfunctions associated with adenomas of the pituitary, the former's study including 73 cases of acromegaly, without adding significantly however to the anatomic data on the genital organs. Reviews of the world's literature on acromegaly (7, 8, 9) and a recent report by Goldberg and Lissner (10) furnish a total of only about a dozen cases in



FIG. 1. The patient.



FIG. 2. Roentgenogram of skull, showing enlargement of sella turcica.

contained large amounts of gonadotropic substance as well as growth hormone. In acromegaly, production of gonadotropin is impaired as the result of the compression of the pituitary basophiles by the eosinophilic tumor.

The present case appears to be the first recorded instance of a granulosa cell tumor of the ovary in a patient with acromegaly. The functional activity of the tumor is attested by the high titer of urinary estrogen four months after cessation of hormonal therapy. This is adequate to explain both the irregular uterine bleeding and the adenomatous hyperplasia of the endometrium. The relation of this endometrial lesion to persistent estrogenic stimulation has been demonstrated in recent papers by Gusberg (12) and Novak and Rutledge (13). Alterations in other endocrine organs, particularly the thyroid, are fairly common in acromegaly; and Gerstel (14) has reported a case of this disorder in which several other endocrine glands were the sites of benign tumors.

The endometrial polyp in the present case may possibly be more than a coincidental finding. Despite the paucity of detailed descriptions of the female pelvic organs, this lesion has been reported in at least 3 other patients with acromegaly, aged 49, 58, and 64 years respectively (6, 10, 15). This is a higher incidence of endometrial polyps than is normally encountered in women of this age group (16) and may signify an etiologic relation to the excessive secretions of the pituitary eosinophiles.

Reece and Leonard (17) have found that estrogen inhibits the effect of growth hormone in the hypophysectomized rat. Although there is nothing in the present patient's history to indicate that remission of her acromegaly was caused by the ovarian tumor, it is interesting that a significant amelioration of her hot flashes, fatigue, and depression were observed for five or six months prior to admission.

SUMMARY

The first recorded instance of a granulosa cell tumor of the ovary in a patient with acromegaly is presented and discussed.

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FIG. 4. Endometrium, showing adenomatous hyperplasia.

which the female reproductive organs are described. These descriptions indicate no specific ovarian pattern in acromegaly. In the elderly women, atrophy was the rule, while in the younger patients cystic changes were common. There was no clinical counterpart of the enormous enlargement of the ovaries which Putman, Benedict and Teel (11) produced in a dog by injection of pituitary extract. It is likely, however, that this extract



FIG. 5. Section of left ovary, showing granulosa cell tumor.

ESTIMATION OF URINARY GONADOTROPIN OF THE NONPREGNANT HUMAN BY THE MOUSE UTERINE WEIGHT AND OVARIAN HYPEREMIA RESPONSES*

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ESTIMATION of urinary gonadotropin excretion for clinical studies usually depends on measurement of the reaction in the gonads of immature rodents caused by administration of extracts of urine, the most widely used methods having as end-points increases in ovarian or uterine weight of rats or mice (1-6). The estimation of gonadotropin by means of measuring its power to increase the weight of the rodent uterus is a relatively sensitive procedure. However, it permits measurement only of the total gonadotropin activity because the estrogen-induced uterine enlargement can be the result either of injected luteinizing hormone or of augmentation of the animal's endogenous luteinizing hormone by administered follicle-stimulating hormone (7, 8). No simple, sensitive and accurate means of differentiating between the gonadotropins present in an extract of urine has been available for clinical use.

Since the ovarian hyperemia response in the rat is an excellent test for urinary chorionic gonadotropin (9), and since this reaction in the rat results from the action of chorionic gonadotropin as a luteinizing hormone (10), it seemed possible that the hyperemia response could be used as the basis of a test for luteinizing hormone in the urine of nonpregnant humans. Farris (11) has reported the production of ovarian hyperemia in the rat by injection of urine obtained from women on the days just preceding ovulation. Riley, Smith and Brown (12) and Kupperman and associates (13) have shown that this reaction is caused by pituitary luteinizing hormone or luteotropin and not by follicle-stimulating hormone. It, therefore, seems probable that the hyperemia reaction following injection of urinary gonadotropin results from the luteinizing hormone and/or the luteotropin content and not from the follicle-stimulating activity. This report presents data on the excretion by humans of gonadotropin as measured a) by the

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centrifuge tubes, aliquots of 1/4, 1/8, 1/16, and 1/32 of the total extract and make up the volume with saline solution to 3 cc. in each tube.

3. Inject subcutaneously 0.25 cc. of solution per mouse twice daily for three days. Injections are at least eight hours apart.
4. Kill animals on fourth day by a blow on the head.
5. Weigh animals to nearest 0.5 Gm. Discard animals which are very heavy or very light. Weight range is from 8 to 15 Gm.
6. Remove ovaries and uterus including cervix. Dissect uterus and ovaries cleanly. Split uterus longitudinally. Blot gently and weigh to nearest 0.1 mg. on torsion balance. Blot gently and weigh ovaries together. A uterine weight to body weight ratio of 2 mg./Gm. or greater is considered positive. Average control ovarian weight is 3 mg. and a weight of over 6 mg. is called positive. At least 4 control animals are killed on each assay day.
7. If the test is positive at all levels run, further serial dilutions are made until a negative level is reached. Normal values: 4-64 m.u.u./24 hours for adult males and females.

D. *Hyperemia assay:*

The 21-day-old mouse has been used in these studies instead of the rat because the hyperemia reaction is at least 10 to 20 times more sensitive in the mouse than in the rat when urinary gonadotropin is administered. Our observations confirm those of Zondek *et al.* (10), who found spontaneous hyperemia reactions more frequent in the mouse. By proper precautions to be described later it has been possible to keep the incidence of these reactions at a very low level which does not interfere with the usefulness of the technique.

1. Dissolve the 24-hour urine extract in a convenient volume of isotonic saline solution (6 cc.).
2. Make up solutions of extract to be given to 4 mice at each dose level. Dilutions routinely used for the initial assay are 1/16, 1/64, 1/128, and 1/256 of the 24-hour extract for each mouse. Each dose is given in one injection in a volume of 1 cc.
3. Give subcutaneously each dilution of extract to one mouse. This is the preliminary "range finding" assay. The results of this "range finding" indicate the dilutions to be administered to 3 additional animals in order to complete the assay on the following day.
4. Kill the mice with carbon monoxide seven hours after injection.
5. Remove the ovaries, including enough of the mesentery and horn of the uterus to prevent drainage of blood from the ovarian veins. The reaction is read by evaluating the color response of the ovary. Three types of reaction are encountered: a) Positive: the ovary is as red as kidney. b) Negative: the ovary is pale white or very slightly pink. c) Equivocal: the ovary is neither as red as kidney nor sufficiently pale to be called "negative." The incidence of these reactions in saline injected mice is as follows:

No. of mice	No. of ovaries	Plus	Plus minus	Minus
127	254	22(8.6%)	95(37.4%)	137(54%)

uterine weight method, which is regarded as an estimate of the total gonadotropin, and b) the ovarian hyperemia method, which is considered an estimate of luteinizing hormone or of luteotropin.¹

METHODS

A. Collection of urine

Forty-eight, or occasionally 24-hour urine specimens, collected without preservative but with the urine stored at ice-box temperature during the collection period, are used.

B. Preparation of extract:²

1. Measure urine volume. Acidify to litmus, filter and add 1 Gm. NaCl per 100 cc. of urine.
2. Add 4 volumes 95% ethyl alcohol; shake well; let stand in refrigerator overnight. Remove supernatant by siphoning and discard. Centrifuge; decant and discard supernatant. Wash precipitate with ether (about 50 cc.). Centrifuge; decant and discard supernatant.
3. Dissolve precipitate in distilled water. Dialyze whole precipitate (against running tap water) in cellophane bag overnight. Pour dialyzed precipitate into centrifuge cup and thoroughly rinse out cellophane bag with distilled water.
4. Centrifuge; decant, saving supernatant. Rinse precipitate and centrifuge again. Pool supernatants.
5. Lyophilize in two equal parts (24-hour aliquots).

C. Total gonadotropin assay:

Two 21-day-old female white mice are used for each dosage level. Total gonadotropin excretion is expressed in terms of mouse uterine units (m.u.u.) per 24 hours. One m.u.u. is that amount of material which will cause an increase in uterine weight of 100 per cent over the uterine weights of saline injected controls. Serial dilutions of the extract are made until the smallest amount of material causing a positive result is found. Ovarian weights are also measured. An ovarian weight increase of over 100 per cent indicates relatively large amounts of F.S.H.

1. Dissolve the 24-hour extract in a convenient volume of isotonic saline solution (6 cc.).
2. Make up solutions of extract to be given to 2 mice at each dose level. The usual beginning assay levels are 8, 16, 32, and 64 m.u.u. Therefore pipette into 15 cc.

¹ We wish to thank Dr. W. W. Westerfeld for his advice in setting up this method.

² The extracts used in this study were prepared by the alcohol precipitation method described. Preliminary studies have shown that the hyperemia-producing material, as well as the gonadotropins which cause a mouse uterine weight increase, can be as effectively extracted from urine by ultrafiltration (8, 14) as by alcohol precipitation. By the use of the ultrafilter, an ovarian hyperemia assay can be completed in less than twelve hours after receipt of the urine specimen.

injection did not increase the specificity of this reaction for luteinizing hormone in a manner such as occurs when other end-points are used (15) and the greater toxicity of the extract when given by this route presented a disadvantage outweighing any possible advantage. Autopsy at seven hours after injection resulted in greater sensitivity than did autopsy at two, four or twenty-four hours after injection.

RESULTS

Normal adults:

The gonadotropin excretion of a normal adult woman during a menstrual cycle as measured by the uterine weight and ovarian hyperemia techniques is depicted in Figure 1. Each point represents the value of half of the gonadotropin contained in a 48-hour urine collection. The necessity

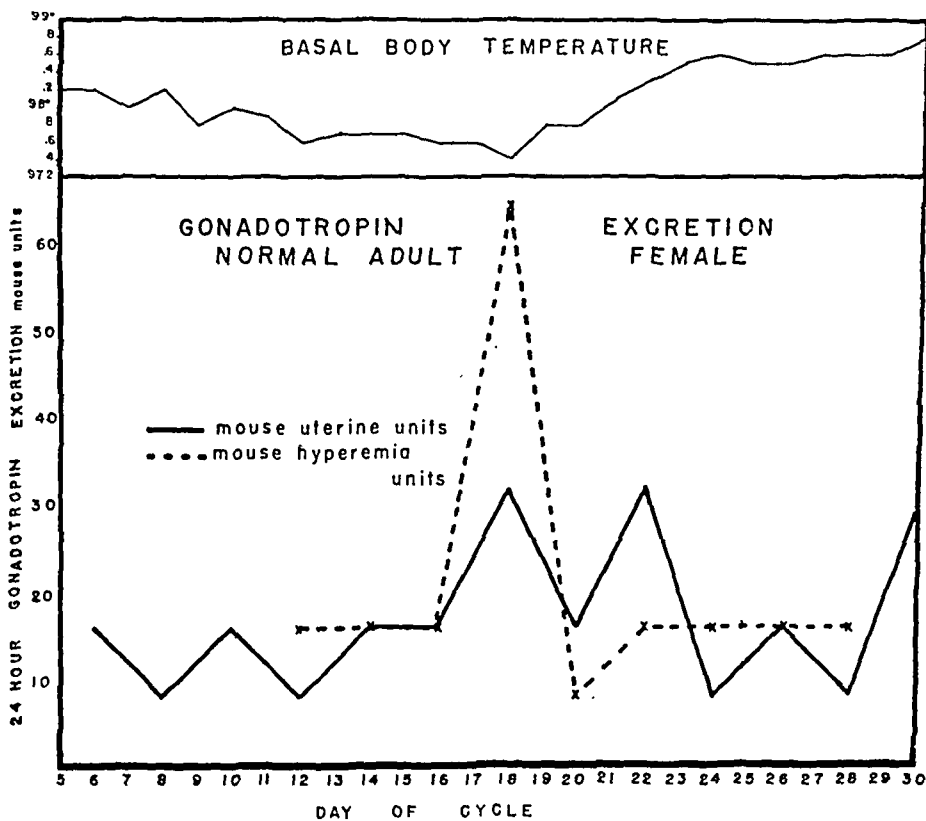


FIG. 1. Gonadotropin excretion of a cyclically menstruating woman throughout one menstrual cycle.

of pooling 48-hour extracts has undoubtedly resulted in decreasing the differences in levels of gonadotropin which might have appeared if daily measurements had been made. Study of two other complete cycles, one in the same subject, the other in a second normal woman, showed the same general pattern of gonadotropin excretion. A rise in total gonadotropin and

A dosage level is called positive when 2 (25%) of the 8 ovaries of the 4 mice receiving that dose show a positive result. A mouse hyperemia unit (m.h.u.) is defined as the amount of extract causing 25% of 8 ovaries to have a "positive" reaction. The "equivocal" reaction is considered "negative" in interpreting the test.

At least 4 saline injected control animals are sacrificed on each day that assays are performed.

Care of animals:

Immature female white mice are used. Animals must be kept at a temperature below 76°F. When animals are raised in an environment warmer than 76°F. a high incidence of "false positive" ovarian hyperemia reactions occurs, even if the animals are kept at below 76°F. for one week before use. These reactions apparently are the result of the high temperature, which causes a generalized vasodilatation, including the vessels of the ovaries.

The size and age of the animals used are factors influencing the incidence of false positive reactions. Animals over 12 to 13 grams in weight and older than 26 days tend to have a higher incidence of "false positive" reactions. Animals which are sick or are extremely small may have decreased sensitivity. We use only animals weighing from 8 to 13 grams, which are healthy and under 26 days of age. These animals are kept in an air-conditioned room at a temperature between 72° and 76°F.

Specificity of the hyperemia reaction:

The hyperemia reaction in the mouse has been caused by the following materials given subcutaneously: sheep pituitary gonadotropin,³ purified prolactin,³ and extracts of urine from normal adult males and females and from castrate males. No reaction was caused by subcutaneous or intraperitoneal injection of extracts of urine from menopausal women (with one exception, when a small amount of hyperemia-producing material was found) and of extracts of urine from 3 patients with pituitary failure resulting from intracranial neoplasms. Intraperitoneal and subcutaneous injection of turpentine, casein (20 per cent) and glucose (25 per cent) in doses as large as could be tolerated by the recipient animals caused no ovarian hyperemia.

Route of administration and time of reaction:

The subcutaneous injection of gonadotropin extract in a single dose produces an ovarian response of magnitude equal to the response following intraperitoneal or subcutaneous injection of divided doses. Intraperitoneal

³ Supplied through the courtesy of Dr. E. C. Reifstein, Jr., of Ayerst McKenna & Harrison, Ltd.

average values of hyperemia-producing material for men are lower than for women. No postcoital increase in hyperemia-producing material in either sex, such as has been reported by Farris (16), has been found.

Normal children:

Two normal boys and 2 normal girls, all in their sixth year, excreted less than 4 m.u.u. per 24 hours and less than 4 m.h.u. per 24 hours.

Hypergonadotropic hypogonadism:

Female: Five patients with ovarian failure either as a result of surgical castration or of physiologic menopause were studied. Uterine weight and ovarian hyperemia values are shown in Table 2. Although gonado-

TABLE 2. GONADOTROPIN EXCRETION OF CASTRATE FEMALES

Subject	Mouse uterine units per 24 hours	Mouse hyperemia units per 24 hours
	Untreated	
1	128	<6
2	128	<4
3	96	<8
4	96	<8
5	32	<5
6	64	16
	Estrogen treated	
7	<8	128
8	<8	256
6		64

tropin excretion as estimated in mouse uterine units was usually high, hyperemia-producing material was low or absent in all patients studied. Three other patients, surgically castrated several years before, were receiving sufficient estrogen at the time of study to produce a marked estrogen effect in the vaginal smear. These patients excreted large amounts of hyperemia-producing material and small amounts of total gonadotropin.

DISCUSSION

A considerable body of evidence indicates that the substance measured by the uterine weight method of gonadotropin assay is different from the material which produces ovarian hyperemia. The uterine weight increase is the result of the total gonadotropin activity present in the urine extract (7, 8). The ovarian hyperemia reaction depends on luteinizing hormone or luteotropin (10, 12, 13). Although the presence of follicle-stimulat-

a proportionately much greater rise in hyperemia-producing material occurred just before the change in basal body temperature considered indicative of ovulation. In Figure 2 are shown representative data concerning the excretion of hyperemia-producing material of other normal women.

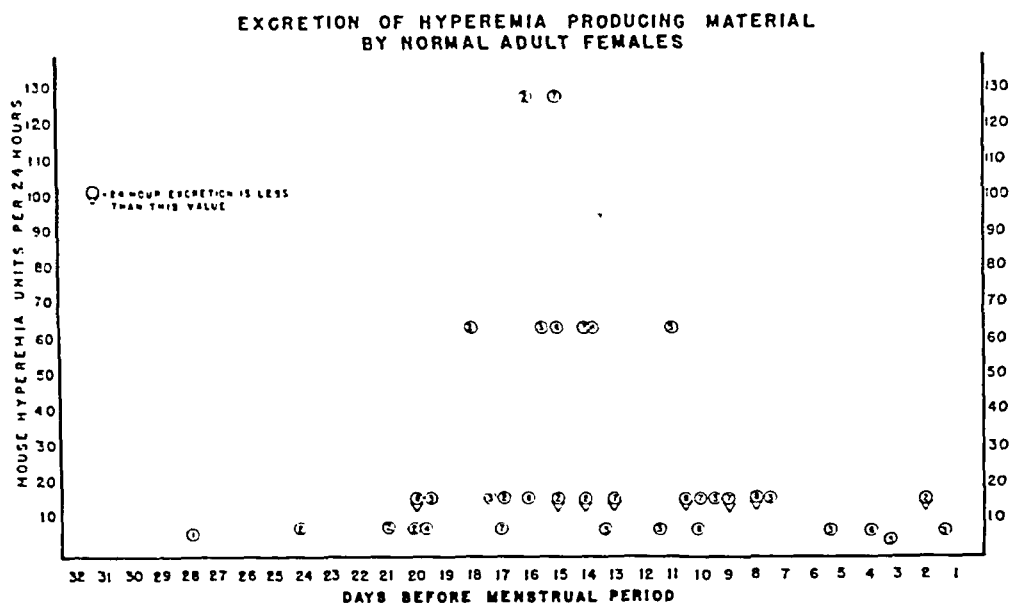


FIG. 2. Excretion of hyperemia-producing material by normal women. Each circle represents the excretion during one 24-hour period. The number within the circle identifies the subject.

Hyperemia-producing material is almost always detectable in the urine of normal women, with large amounts being present at the time of ovulation. The material is less frequently found in the urine of normal males, but is often detectable. Values for normal men are shown in Table 1. The

TABLE 1. GONADOTROPIN EXCRETION OF NORMAL ADULT MALES

Subject	Mouse uterine units per 24 hours	Mouse hyperemia units per 24 hours
1	<8	16
2	<8	< 4
3		<16
4	16	8
5		16
6	8	<16
7		<16
8	<8	<16
9	<8	<16

castrate women who are receiving sufficient estrogen to produce a definite vaginal smear effect, contains hyperemia-producing material in amounts as great or greater than is found at the time of ovulation in the urine of normal women. Further studies of the urinary excretion of gonadotropin by castrate males and females and of the effects of estrogen on gonadotropin excretion will be published in a separate report.

SUMMARY

1. A method is described for measuring the urinary excretion of material which is capable of causing ovarian hyperemia when injected into the immature mouse. Mouse uterine weight assays were also performed on the same specimens.

2. The assay which measures the amount of ovarian hyperemia-producing material is considered to be an estimation of luteinizing hormone or luteotropin excretion.

3. The assay which utilizes the uterine weight increase as an end-point is considered to be an estimation of total gonadotropin excretion.

4. The normal woman excretes at all times material which causes an increase in the uterine weight of the mouse and which produces an ovarian hyperemia. Ovulation is preceded by a moderate increase in total gonadotropin and by a proportionately much greater increase in hyperemia-producing material.

5. The normal male excretes less of the hyperemia-producing material than is excreted by the normal female.

6. The castrate female excretes large amounts of total gonadotropin and very small amounts of the hyperemia-producing material. Administration of estrogen in quantities sufficient to cause a considerable estrogen response of the vaginal smear is followed by excretion of small amounts of total gonadotropin and large amounts of hyperemia-producing material.

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ing hormone slightly augments the ovarian hyperemia-producing activity of luteinizing hormone, the degree of augmentation is not great (13). For clinical assays this augmentation is disregarded. In our studies, the uterine weight method of assay is considered as a measure of the total gonadotropin present in the extract, and the hyperemia-producing material is considered to be either luteinizing hormone or luteotropin. No method is available for differentiation between luteinizing hormone and luteotropin, so that it is impossible to decide what portion of the hyperemia-producing activity of an extract depends on each of these hormones.

If the hyperemia-producing material found in urine represents luteinizing hormone, its excretion by the normal adult is to be expected. The presence of luteinizing hormone is required for the secretion of gonadal steroid hormones (7). The normal adult is never completely without gonadal hormones and, therefore, some small amount of luteinizing hormone must be present at all times. In the female, the increase in hyperemia-producing material precedes ovulation and probably represents the luteinizing hormone which causes ovulation. We have found a high peak of excretion at the time of ovulation when urine concentrates are used. We have not been able to confirm the observation of Farris (11) that sufficient hyperemia-producing material is excreted before ovulation to produce a positive response when unconcentrated urine is injected.

Levin and Tyndale (1) and Leatham and Levin (17) have likened the gonadotropin found in urine of normal males to that found in the urine of castrate females, reporting that the urine of males has a high content of follicle-stimulating activity and a small amount of luteinizing activity. In our study, the average of values of hyperemia-producing material in urine extracts was less in males than in normal females, but was greater than for castrate females. The average total urinary gonadotropin was somewhat less in normal males than in normal women and was much less than the total gonadotropin excretion of castrate women.

The castrate women included in this study excreted large amounts of total gonadotropin and small amounts of hyperemia-producing material. In this type of patient the total gonadotropic activity consists principally of follicle-stimulating hormone (1, 2, 18, 19, 20, 21). The low values for luteinizing hormone determined by the hyperemia method are in agreement with the low values found by other means for detecting luteinizing activity. Although the luteinizing activity is low, definite though slight hyperemia-producing effects are sometimes obtained with urine from castrates. Other laboratories have also reported data indicating that the gonadotropin in the urine of castrates is not a pure follicle-stimulating hormone but also contains small amounts of luteinizing hormone (22). Preliminary studies, as indicated in Table 2, suggest that the urine of

THE EXCRETION OF NEUTRAL LIPID-SOLUBLE REDUCING SUBSTANCES BY INFANTS*

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Denver, Colorado*

THE development of chemical methods (1, 2, 3) for the measurement of adrenocortical steroids in urine has made possible the evaluation of the level of excretion of these substances in normal individuals and in relation to diseases known to involve the adrenal cortex. A considerable amount of data is now available concerning the normal level of excretion of adrenocortical steroids by adults. Little has been done toward the determination of their elimination by infants. Knowledge of the excretory level of these compounds during the first few weeks of life may aid in explaining the function of the human fetal adrenal cortex. In order to estimate the level of excretion of adrenocortical steroids in normal infants and in the hope that these data might help toward the evaluation of the function of the fetal cortex, the amount of neutral lipid-soluble reducing substances excreted in the urine of premature and full-term infants was determined during the period when involution of the fetal cortex is known to occur.

METHODS

Twenty-four hour urine specimens were collected from babies in the Premature Center and the Pediatric Ward of the Colorado General Hospital.¹ The urine was collected without preservative. It was stored in the refrigerator and was analyzed within twenty-four hours after collection. The determinations of neutral lipid-soluble reducing substances² were done by the method of Heard, Sobel and Venning (2) and the results are expressed in terms of desoxycorticosterone³ as a standard. Analyses per-

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* From the thesis presented to the Graduate School of the University of Colorado, by Charles F. Matscn, in partial fulfillment of the requirements for the M.S. degree.

¹ We gratefully acknowledge the cooperation of Dr. Clifton D. Govan and staff members of the Department of Pediatrics who collected the urine specimens. The method of Hoag (4) was used for the urine collections.

² The method used is not specific enough to identify these substances unequivocally as adrenal cortical compounds.

³ We wish to express our thanks to Dr. C. R. Scholz of Ciba Pharmaceutical Products, Inc., who furnished desoxycorticosterone.

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has reported values for the excretion of these substances by infants. The excretory levels varied from 0 (no reduction) to 0.2 mg. per 24 hours as measured by the method of Talbot, Saltzman, Wixom and Wolfe (1). These values are considerably lower than those reported here, but the method used by Day characteristically gives lower results than does the method of Heard, Sobel and Venning (2). Venning and Kazmin (6) were unable to detect any glycogenic activity in the urine of newborn male infants during the first four days of life.

In the present work there was no instance in which the urine failed to contain lipid-soluble reducing substances. In fact, the highest value observed, 0.62 mg., was excreted by a full-term infant 4 days of age. The smallest quantity, 0.17 mg., was excreted by a premature infant 3 days of age. The latter value is questionable because the small quantity of urine prevented duplicate determinations. Although the amount of data is too small to be certain, there appears to be no correlation between the age of the infant and the quantity of lipid-soluble reducing substances excreted. Neither does the amount excreted seem to depend upon whether or not the infant was born prematurely.

The babies in this study excreted much less of the lipid-soluble reducing substances than do adults. On a body weight basis, however, adults excrete approximately 0.02 mg. per Kg. per day, whereas these infants excreted approximately 0.12 mg. per Kg. per day. This would seem to indicate that the gland at this age is working at a higher level than that of the adult, although it is possible that the substances measured by this procedure may originate, in part, in locations other than the adrenal cortex. As Day (5) has pointed out, if one compares the level of excretion to the weight of the adrenal gland, this difference between the infant and the adult probably would disappear. However, Sayers and Sayers (7) emphasize that the level of secretion of the adrenal cortex is determined, in part at least, by the demand for the hormones that exists in the tissues. If this is the case, the expression of the level of excretion of the adrenocortical steroids in terms of body weight is perhaps a good method of comparison. On this basis our data indicate a greater cortical secretion in infants than in adults.

The question of the function of the human fetal adrenal cortex remains an enigma. The results herein reported might be taken to indicate that the fetal cortex produces substances similar to those produced by the adult cortex, and a larger quantity of them. On the other hand, the apparently greater secretion might be the result of the loss of stored materials occasioned by the involution of the fetal cortex. Certainly these data do not give any precise information concerning the function of this interesting organ.

formed on urine from adults gave results between 1.04 and 2.54 mg. per 24 hours, in comparison to the range of 1.1 to 2.1 mg. reported by Heard, Sobel and Venning (2). Recovery experiments with desoxycorticosterone were satisfactory.

RESULTS

All of the babies examined were males. Twelve determinations on urine specimens from 8 premature infants and 5 determinations on specimens from 5 full-term infants were made. The results are recorded in Table 1.

TABLE 1. THE URINARY EXCRETION OF LIPID-SOLUBLE REDUCING SUBSTANCES PER 24 HOURS BY INFANTS*

	Subject	Birth weight	Weight at time of collection of urine	Age at time of collection of urine	Urine volume per 24 hours	Lipid-soluble reducing substance per 24 hours
		Gm.	Gm.	days	ml.	mg.
<i>Premature Infants</i>	1.	2,119	2,041	3	49	0.17
			2,112	9	87	0.29
			2,182	15	57	0.30
	2.	2,140	2,098	13	50	0.20
			2,404	20	116	0.34
	3.	1,911	2,098	9	130	0.38
	4.	2,240	2,249	12	350	0.22
	5.	2,232	2,183	15	113	0.27
<i>Premature Infants</i>	6.	2,017	2,313	25	†	0.27
			2,495	28	211	0.36
	7.	1,903	2,580	27	112	0.20
	8.	1,389	2,108	33	147	0.22
<i>Full-term Infants</i>	9.	3,048	2,835	5	155	0.29
	10.	3,300	3,473	5	105	0.26
	11.	3,600	3,459	4	320	0.62
	12.	3,147	3,090	6	164	0.31
	13.	3,441		17	232	0.28

* Referred to desoxycorticosterone as a standard.

† Part of sample lost. Value given is 0.27 mg. per 150 ml.

The amounts of lipid-soluble reducing substances excreted by the premature infants varied from 0.17 mg. to 0.38 mg. per 24 hours. The full-term infants excreted a minimum of 0.26 mg. and a maximum of 0.62 mg. per 24 hours.

DISCUSSION

The results show that measurable amounts of lipid-soluble reducing substances are excreted by both premature and full-term infants. Day (5)

Letter to the Editor

TO THE EDITOR:

HYPERSENSITIVITY TO PITRESSIN

I SHOULD like to take this opportunity to write to you concerning the development of hypersensitivity to pitressin (beta-hypophamine) in the treatment of idiopathic (primary) diabetes insipidus. Such a situation occurred in a 33-year-old female who was receiving pitressin therapy for known diabetes insipidus.

This patient had been given one or two nasal tampons saturated with 1 to 2 cc. of pitressin in aqueous solution as required to control the diuresis for approximately two and one-half years. Gradual failure to influence the diuresis necessitated discontinuation of this form of therapy, and the intramuscular injection of 1 cc. of pitressin tannate in oil daily was instituted. After one month of using this route, erythematous areas appeared over the injection sites. On several occasions there occurred malaise, general aching, and a feeling of stiffness in the hands, feet and lips. There was one episode with some vomiting and tetany with generalized clonic spasms and marked carpopedal spasm.

An intracutaneous test with an aqueous solution of pitressin produced a pruritic, erythematous, elevated wheal 4.5 cm. in diameter.

The administration of 400 to 500 mg. of benadryl (diphenhydramine hydrochloride) daily has prevented local and systemic symptoms of allergy and has permitted the administration of the quantity of pitressin tannate required to control the diuresis and polydipsia. However, pressure urticaria at sites where constricting clothing presses against the body is a persistent, troublesome symptom.

When there has been a deficient intake of the antihistamine drug, without change in the amount of pitressin tannate, systemic symptoms have been presaged by alkalinity of the urine. Fifteen to 30 grains of ammonium chloride gave the urine a neutral reaction, abolished the malaise, aching, and stiffness; and nullified the antidiuretic action of the pitressin tannate.

When the pitressin tannate was given with a single, simultaneous dose of benadryl (100 mg.) and blood studies were done ten hours later (the usual time interval between giving the pitressin and the onset of untoward symptoms), the levels of calcium, phosphorus, albumin, globulin, total proteins and A/G ratio were all within normal limits.

The appearance of bizarre symptoms in individuals receiving continuous pitressin therapy should make one alert to the possibility of the development of hypersensitivity to this substance.

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JOHN BRICE PLASS, M.D.
January 4, 1949.

SUMMARY

The lipid-soluble reducing substances have been determined in the urine of premature and full-term infants. The total quantity of material excreted was low but, per unit of body weight, was higher than that excreted by adults. The possible bearing of the results on the problem of the function of the fetal adrenal cortex is discussed.

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2. Nor-Epinephrine in Adrenal Medulla.

Marcel Goldenberg and Mogens Faber (introduced by R. F. Loeb).

From the Departments of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y.

Studies on the hemodynamics of epinephrine and nor-epinephrine and its possible relation to the problem of hypertension have raised the question as to whether nor-epinephrine is present in the normal adrenal medulla and in chromaffin tissue tumors (pheochromocytomas).

Since bioassay of small amounts of nor-epinephrine in mixtures containing large amounts of epinephrine is not feasible, chemical methods were used to show the presence of nor-epinephrine in extracts of the adrenal medulla of cattle.

A good separation of nor-epinephrine from epinephrine can be obtained by paper chromatography using phenol as solvent (James). By modifying James' method, a sensitivity as low as 2 micro g. could be obtained and a quantitative estimation by planimetric measurement of the spot areas was possible.

Extracts of the adrenal medulla of cattle (epinephrine U.S.P. reference standard) were found to contain up to 18 per cent nor-epinephrine.

The epinephrine fractions from 3 chromaffin tumors were examined and found to contain 50 to 90 per cent nor-epinephrine.

These findings are of interest since epinephrine and nor-epinephrine differ significantly in their pharmacologic actions. Recent hemodynamic investigations in man using the method of right heart catheterization have shown that epinephrine within a physiologic range acts as an overall vasodilator and causes hypertension only by increase of cardiac output. Nor-epinephrine, on the other hand, acts as overall vasoconstrictor with no change or slight decrease of cardiac output. These two agents also differ in their actions on metabolism; *e.g.*, the hyperglycemic action of nor-epinephrine is much less marked than that of epinephrine (ratio 1:8).

3. Studies on an Anti-Diuretic, Non Chloruretic Substance Extracted from Urines of Normal and Cirrhotic Subjects.*

Elaine P. Ralli, Stephen Leslie (by invitation), George H. Stueck, Jr. (by invitation), Mary E. Dumm and Bertram Laken (by invitation).

From The Laboratories of the Department of Medicine, New York University College of Medicine.

The method of extracting the anti-diuretic substance from urine has been modified as follows: 24-hour urine samples divided in aliquots of 200 cc. contained in Visking cellulose casing (36/32 Nojax) were dialyzed for two hours against 3 changes of distilled water. The N and Cl concentration of the urine decreased during dialysis to less than 10% of the original level. The urines were concentrated about 25 times, pooled, and chromatographed, using a permutit column, which was eluted first with distilled water and then with 5% NaCl in 1 M acetic acid.

Na, Cl, N and arginine were determined in the eluates. Anti-diuretic activity was estimated by the bioassay method of Burn. No significant anti-diuretic activity was present in the distilled water eluates. However, large amounts of A. D. S. were found in the NaCl-HAc eluates.

The urine chlorides were determined during the period of bioassay and compared to

* This research was aided by a grant from the United States Public Health Service, Research Grants Division.

THE ASSOCIATION
FOR THE STUDY OF
INTERNAL SECRETIONS

ABSTRACTS OF PAPERS PRESENTED
AT THE THIRTY-FIRST ANNUAL MEETING

FRIDAY AND SATURDAY, JUNE 3 AND 4, 1949

Haddon Hall

ATLANTIC CITY, NEW JERSEY

Scientific Sessions in the Viking Room

(Abstracts arranged according to number on printed
program.)

FRIDAY, JUNE 3, 1949

9:15 A.M.—C. N. H. LONG, PRESIDING

1. Piperido-methyl-benzodioxane (933-F): Some Pharmacological and Experimental Observations.

Evan Calkins (by invitation), George W. Dana (by invitation), J. C. Seed (by invitation) and John Eager Howard, M.D.

From the Department of Medicine, The Johns Hopkins University and the Army Chemical Center, Edgewood, Maryland.

Piperido-methyl-benzodioxane (933-F) reversibly opposes the pressor and most of the other effects of epinephrine and allied compounds by competition for the epinephrine-specific receptor substance. Comparison of the formulae of the benzodioxanes, yohimbine, and the other reversible epinephrine inhibitors indicates that all these substances have the C-O-C-C-N group in common. Dibenamine, however, which irreversibly blocks the pressor action of epinephrine, lacks the oxygen atom in this group. It presumably combines chemically with the receptor substance at this point.

Sharp reduction in blood pressure following the intravenous injection of approximately 20 mg. of 933-F has been reported in 4 cases of pheochromocytoma. The present authors find similar responses, not only in a patient with pheochromocytoma, but also in a patient with neuroblastoma. Both patients were relieved of their hypertension following surgical removal of the tumors, although in the second case the hypertension returned with the development of liver metastases.

The blood pressure responses and side effects following administration of 933-F to over 100 patients with hypertension from other causes are described. In none of these was there a significant fall in blood pressure or any other ill effects. 933-F appears to provide a safe and effective clinical test for an excess of circulating epinephrine or epinephrine-like substance.

other although the BMR was normalized. Thyroid hormone and TSH were present in excess in acromegaly. Normal sera possessed thyroid hormone activity with either minimal or no TSH present.

The results show that fluctuations in the thyroid-thyrotropic hormone balance characterize the circulating fluids in thyroid and pituitary gland disturbances, and that these can be detected in the tadpole with as little as 0.35 cc. of untreated serum.

6. Estimation of Urinary Gonadotropin of the Nonpregnant Human by the Mouse Uterine Weight and Ovarian Hyperemia Responses.

Charles W. Lloyd, Muriel Morley (by invitation), Kathryn Morrow (by invitation), Julia Lobotsky (by invitation) and Edward C. Hughes (by invitation).

From Syracuse University College of Medicine, Syracuse 10, New York.

A study has been made of urinary excretion of material capable of causing ovarian hyperemia when injected into immature mice. Mouse uterine weight assays were performed on the same specimens. The material producing ovarian hyperemia is considered to be either luteinizing hormone or luteotropin. The uterine weight assay is thought to estimate total gonadotropin excreted.

Normal women excrete at all times uterine weight-increasing and ovarian hyperemia-producing materials, with a moderate increase in total gonadotropin and a proportionately much greater increase in hyperemia-producing material at ovulation. Less hyperemia-producing material is excreted by normal men.

Female castrates excrete large amounts of total gonadotropin and small amounts of hyperemia-producing material. Estrogen therapy causes a reversion toward the normal pattern with production of large amounts of hyperemia-producing material.

The urine of castrate males contains large amounts of total gonadotropin and hyperemia-producing material. Androgen therapy causes decrease of hyperemia-producing material to normal levels and estrogen therapy does not increase excretion of this substance. A patient with Klinefelter's syndrome had high total gonadotropins but normal hyperemia-producing material.

It is suggested that fundamental differences exist in function and secretory control between the anterior pituitaries of adult human males and females.

7. Further Studies of Antigonaotropin Formation in Man.

James H. Leatham and A. E. Rakoff.

From the Bureau of Biological Research, Rutgers University, New Brunswick, N. J. and the Department of Obstetrics and Gynecology and the Endocrine Division, Jefferson Medical College and Hospital, Philadelphia.

Of 42 patients treated with a combination of sheep anterior pituitary and chorionic gonadotropin (Synapoidin), 5 developed antihormones. All were patients with functional menstrual disorders. The antiserum was nonspecific in that it antagonized the gonadotropic action of chorionic gonadotropin and pregnant mare serum.

Equine pituitary gonadotropin (Squibb) studies have been extended to 26 patients, 12 of whom developed antihormones. The gonadotropin was usually administered in 50 unit dosages two to three times weekly but for only two weeks of each month in females. Unlike the antisera obtained from rabbits treated with equine pituitary, the anti-gonadotropic action of human serum is not specific. The human anti-equine pituitary sera counteracted the gonadotropic action of pregnant mare serum in 8 of 8 cases, of chorionic gonadotropin in 7 of 8 cases and of sheep pituitary plus chorionic gonadotropin in 2 of 6 cases. The action of human pituitary material was not influenced. In 3 cases no

the urine chlorides following the injection of commercial pitressin in doses of equivalent anti-diuretic activity. Although the eluates had a marked anti-diuretic effect, the chloride concentration was not increased (16-21 mEq./L.), whereas commercial pitressin had a marked chloruretic effect (42-112 mEq./L.).

4. A Method for the Assay of Prolactin in Human Urine.*

Richard L. Coppedge (by invitation) and Albert Segaloff.

From the Departments of Physiology and Medicine of Tulane University and The Alton Ochsner Medical Foundation of New Orleans, Louisiana.

Prolactin is extracted from 24-hour urine specimens by acid-alcohol precipitation and dialysis against 0.5% saline. Assay by the local method of Lyons, as modified by Hall, is not entirely satisfactory because the prolactin response is often obscured by a non-specific inflammatory reaction. Systemic assay, employing our intravenous method, gives results which correlate satisfactorily with those of local assay. In both methods, pigeons are injected daily for four days and sacrificed on the fifth day and the crop observed.

Local assays on 4 normal women revealed a urinary prolactin excretion (as I.U./24 hrs.) in the first subject of <25; in a second, on 3 specimens, (1) <50 and 20, (2) 25 and 20, (3) >25 <50; in a third, on five specimens, (1) 100, (2) 100, (3) 60, (4) <50, (5) >50 <100 (S7 by intravenous method); and in a fourth, >25 <100. One normal male showed values of 50 and 100, and another >50 <100.

Local assay of urines with added prolactin reveal values proportional to the amount of hormone added.

Assays have been made in patients with breast carcinoma, chronic cystic mastitis, fibromyomata uteri, endometriosis, precocious puberty, pituitary tumor, Cushing's syndrome (adrenal carcinoma) and pre- and post-partum. As yet, the level of prolactin excretion cannot be definitely correlated with the pathology or with other endocrine assays.

5. Thyrotropic and Thyroid Hormone Assay of Normal and Pathologic Human Sera in the Stasis Tadpole.

S. A. D'Angelo, A. S. Gordon, K. E. Paschkis and A. Cantarow.

From the Department of Biology, Washington Square College of Arts and Science, New York University and Jefferson Medical College, Philadelphia, Pa.

It has been demonstrated previously that the stasis (starved, non-metamorphosing) tadpole is a sensitive test object for the detection of thyrotropic hormone and thyroxine, and that the resulting metamorphosis with either agent can be differentiated by direct examination of the thyroid in the test animal (D'Angelo, Gordon, and Charipper, *Endocrinology* 31: 217, 1942). The present experiments indicate that the method can be used to determine the status of thyroid-thyrotropic balance in the blood of normal individuals and those with endocrine disorders. Five to seven injections of 0.5 cc. of untreated serum, given on alternate days, advanced metamorphosis in the test animal. Correlation of the developmental changes with presence or absence of thyroid gland activation revealed that the thyroid-TSH equilibrium in the blood is shifted in endocrinopathies. In hypothyroidism (2 cases) serum was high in TSH but low in thyroid hormone. In thyrotoxic patients (2 cases) thyroid hormone was high with no TSH detectable. TSH was high in the blood of euthyroid individuals with moderate or severe ophthalmopathy (2 cases). Thyroid medication reversed the thyroid-TSH balance in one patient but failed in an-

* This work was supported in part by a grant from the National Institute of Health and in part by a grant from the National Research Council, Committee on Endocrinology.

10. Regulation of Pituitary Adrenocorticotrophic Activity by Adrenal Cortical Hormones.

Chi-Ping Cheng (introduced by George Sayres).

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

The content of adrenocorticotrophic hormone (ACTH) in the pituitary of the rat is reduced to 20% of normal 24 hours after adrenalectomy. In animals with intact adrenals, nonspecific stress (scald, shamadrenalectomy) reduces the ACTH content of the pituitary by 50%. Pretreatment of adrenalectomized rats with desoxycorticosterone acetate (DCA) prevents the depletion which follows adrenalectomy (pituitary ACTH content, 43% of normal). The results lend additional support to the concept that pituitary adrenocorticotrophic activity is regulated by the level of cortical steroids in the body fluids.

DCA in the adreno-demedullated rat produces a state of insulin hypertension which can be corrected by the concomitant administration of whole adrenal cortical extract. DCA elevates plasma sodium and decreases the excitability of the central nervous system; neither phenomenon occurs in rats given ACTH or whole extract of the adrenal cortex together with DCA. These results may be interpreted to mean that DCA inhibits the release of ACTH from the pituitary and depresses the endogenous production of cortical hormones; a steroid hormone imbalance develops which is characterized by an excess of DCA (salt regulating) and a deficiency of the carbohydrate regulating steroids.

11. Adequacy of Pituitary Adrenocorticotrophic Function in Nutritional Deficiencies.

George Sayers.

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

Two indices have been employed to appraise the functional activity of the pituitary-adrenal system of rats with chronic malnutrition (low-protein diet). Content of adrenocorticotrophic hormone (ACTH) in the pituitary was measured by direct assay; rate of release of ACTH, by adrenal size and chemistry. Malnourished rats had approximately the same large pituitary store of ACTH as normal rats. The adrenals of the low-protein rats were smaller than normal and had a greater concentration of cholesterol, indicating reduced adrenal cortical activity, probably in response to reduced body need for cortical steroids. After scalding, the content of ACTH in the pituitary at 24 hours was reduced approximately 50% in both groups. The content was restored to normal in both groups at 48-72 hours, despite continued ACTH discharge. The capacity to discharge ACTH was not appreciably altered in the malnourished rats as judged by the increase in size of the adrenals and the reduction in adrenal cholesterol and ascorbic acid after scalding. Malnutrition affects the functional capacity of the pituitary-adrenal system only slightly; this is in contrast with its marked effect on the pituitary-gonad system.

12. Effects of Prolonged Adrenal Cortical Stimulation upon Free and Esterified Serum Cholesterol in Normal Men.

Jerome W. Conn, and William C. Vogel (by invitation).

Ann Arbor, Michigan.

In 3 separate experiments upon 2 normal men, 56 duplicate determinations of free and ester cholesterol (Shoenheimer-Sperry technic) were made upon the sera before, during and after a course (6-8 days) of large amounts of ACTH. The response was as follows: 1) A mild decrease of the ester fraction on the second day of ACTH with no

anti-equine pituitary action was obtained but the same sera nullified the gonad stimulating action of pregnant mare serum.

S. The Evaluation of the Use of Anterior Pituitary Extract in the Treatment of Pituitary Dwarfism.

Joseph C. Edwards, Cecil M. Charles (by invitation) and Cyril M. MacBryde.

From the Department of Internal Medicine, Washington University School of Medicine, Saint Louis, Mo.

Eleven patients with pituitary dwarfism were studied and treated for periods ranging from one to six years. Control periods were employed before and at intervals during treatment with a pituitary extract containing the growth principle.

Method of Study:—After preliminary periods of study with accurate anthropometric measurements each of the 11 patients was given "Phyone" (Wilson) pituitary extract which contains both growth and thyrotropic factors. Graduated doses increasing up to 2 cc. thrice weekly were continued for two to six months with control intervals without treatment. Eight of the patients were given no thyroid, androgen or estrogen during their observations on pituitary extract treatment.

Results:—The height of only one of the 11 patients exceeded at the end of treatment the minimum normal for his age. The normal growth curves for boys and girls are shown in the same scale as the growth curves of this group, for comparison. Our patients failed to show an increase in stature or weight so definite that it could be attributed to the use of growth extract "Phyone" alone. In the treatment of dwarfism due to primary hypopituitarism, pituitary extract therapy alone is, at present, unsatisfactory. Failure to obtain a growth response in human beings has recently been demonstrated not only with the commercially available products but also with relatively large doses of the pure growth principle. The lack of response may result from interfering factors in patients or from inadequate amounts of the growth principle in the preparations administered.

9. On the Inability of Adrenocorticotrophic Hormone or Epinephrine to Deplete the Ascorbic Acid of the Chick Adrenal.

Norman F. Boas (by invitation) and Joseph W. Jailer.

From the Endocrine Section of the Medical Services, and the Chemical Laboratory of The Mount Sinai Hospital, New York.

Long, Sayers, and their collaborators have demonstrated that the administration of ACTH results in a prompt fall in adrenal ascorbic acid and cholesterol. Exposure of animals to stress (*e.g.*, epinephrine, histamine, cold) also results in a decrease in adrenal ascorbic acid and cholesterol in the normal, but not in the hypophysectomized rat. In the light of these observations it has been proposed that the depletion of ascorbic acid is an index of the elaboration of adrenal corticosteroids. This fall in adrenal ascorbic acid has been demonstrated in the rat and guinea pig. We have observed that such is not the case in the chick. The adrenals of White Leghorn cockerels from 2 to 41 days of age were examined. Shortly after hatching, the ascorbic acid concentration of the adrenals was 117 mg. %. This increased to a maximum of 201 mg. % at 41 days, in roughly a straight line curve. Following the administration of 25 mg. of ACTH (33rd and 41st days) and epinephrine (14th, 18th, and 41st days), no alteration in the adrenal ascorbic acid content or concentration was produced. The implications of these observations are discussed.

new carbohydrate, other than from the tissues or injected protein, determined whether protein catabolism would be stimulated by ACE. In the present investigation the need for new carbohydrate was accentuated by insulin hypoglycemia. It was found that insulin produced a small increase in urea formation in the first three hours and a larger increase in the second three hours after injection, but when preceded by two hours by 1.0 ml. of ACE per 100 grams body weight a three to four fold increase in urea formation occurred during the first three hours compared to animals receiving ACE or insulin alone. Prevention of insulin hypoglycemia by glucose completely abolished the increase in urea formation. The significance of these findings with respect to the mechanism of the protein catabolic action of the adrenal cortex and the possible role of stress per se in this phenomenon is considered.

15. Renal Function in Normal and Adrenalectomized Rats Following Saline or Adrenal Steroid Administration.

W. R. Boss (by invitation), James H. Birnie and Robert Gaunt.

From the Department of Zoology, Syracuse University, Syracuse, N. Y.

Studies of renal function in untreated adrenalectomized rats indicating that decreased glomerular filtration may contribute to the loss of water diuresis, but that an increase in tubular reabsorption is probably a more important factor, have been reported by the authors.

Creatinine clearance studies were conducted on female normal and adrenalectomized rats weighing approximately 175 grams. Sixty-three rats divided between the various groups studied were used in this investigation. The glomerular filtration rate (C-Cr) of normal rats given two doses of water (3 cc./100 sq. cm. body surface/dose) by stomach tube was .956 cc./100 Gm./minute and the urine flow (V) was .062 cc./100 Gm./minute. In rats adrenalectomized for seven days and maintained on a 1.0 per cent saline solution as drinking fluid the C-Cr was .738 and the V was .007. Rats adrenalectomized for seven days and maintained by daily subcutaneous injections of 0.5 mg. DCA had a C-Cr of .844 and a V of .018. These results indicate that while saline (as found by Lotspeich) or DCA will maintain essentially normal glomerular filtration in adrenalectomized rats the urine volume remains far below normal, presumably due to an elevated tubular reabsorption of water.

When 5.0 mg. of DCA was administered to intact rats the C-Cr was .900 and the V .077. Following the oral administration of 4 cc. of cortical extract (Upjohn) to intact rats the C-Cr was .993 and the V .076. The increase in the rate of urine flow without an accompanying rise in glomerular filtration in overdosage experiments is interpreted as indicating that these adrenal steroids cause a decrease in the tubular reabsorption of water. Our work with DCA confirms that of Winter and Ingram in dogs.

16. Adrenal Cortical Hormone in Blood.

K. E. Paschkis, A. Cantarow and D. Boyle (by invitation).

From Jefferson Medical College, Philadelphia, Pa.

Levels of adrenal cortical hormone were determined in the blood of dogs, using the mouse-liver-glycogen method of E. Venning. Small amounts of hormone were present in the arterial plasma of normal dogs. Injection of ACTH or of epinephrine caused a prompt rise of hormone levels.

A rise also occurred after subcutaneous injection of formalin (alarming stimulus). Intravenous injection of insulin was followed by an increase of cortical hormone levels, suggesting the participation of cortical hormone in counteracting insulin-induced hyperglycemia.

change of free cholesterol, 2) Beginning on the third day and becoming more intense with time, a sharp fall of the ester fraction (maximal fall 77 mg. % or 45% below baseline), 3) During this same period, a fall of free cholesterol (max. 17 mg. % or 28% below baseline), and 4) Upon cessation of ACTH, prompt rebound of free cholesterol to or above the baseline value (1-2 days), whereas it required six days of gradually increasing values before the ester fraction returned to baseline.

Although two less likely explanations exist, we interpret the data as indicating 1) that under forced stimulation of the adrenal cortex the blood constitutes an important source of material for synthesis of cortical steroids, 2) that esterified cholesterol is the immediate precursor of the steroidal hormones, and 3) that increased cholesterol synthesis can more easily keep pace with the increased need for this basic compound than can the process by which it is esterified.

13. The Possible Involvement of the Adrenal Cortex and Thyroid in Mobilization of Fat to the Liver.

Louis Levin.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Previous work has demonstrated that marked increase in the liver fat level may be induced in mice by administration of suitable pituitary extracts or by subjection to some, but not all, physical stresses. The present work consists of experiments attempting to elucidate the mechanism whereby liver fat is so rapidly mobilized.

Mice adequately treated with thiouracil respond to stress equally as well as do untreated mice, indicating that thyroidal activity is not necessary for liver fat mobilization. Crude pituitary extracts or pure adrenotropic hormone (Armour), though very effective in causing fat mobilization in intact or in shamadrenalectomized mice, are completely inactive in this respect in adrenalectomized mice. Adrenalectomy, without further treatment, results in slight loss of liver fat. Exposure of adrenalectomized mice to physical stress does cause a slight increase in liver fat content but the increase is of much smaller magnitude than that elicited by similar treatment of intact animals.

Administration of 17-hydroxycorticosterone, 11-dehydrocorticosterone and 11-deoxycorticosterone to adrenalectomized mice causes a slight increase in the reduced liver fat level but never enough to surpass that of untreated intact mice.

Bile secretion in mice is apparently completely inhibited by adrenalectomy but may be re-induced by treatment with suitable adrenocortical steroids.

2:00 P.M.—J. S. L. BROWNE, PRESIDING

14. Stimulation of Nitrogen Metabolism by Adrenal Cortical Extract During Insulin Hypoglycemia.

Frank L. Engle.

From the Department of Medicine, Duke University School of Medicine, Durham, N. C.

In previous studies it has been shown that the rate of urea formation in the nephrectomized rat could be used as a sensitive measure of the rate of protein catabolism during brief periods of observation. It was found that adrenal cortical extract (ACE) increased urea formation, this increase being prevented by intravenous glucose or amino acids two hours after the ACE but not by a fat emulsion or serum albumin similarly administered. Evidence was presented that the action of ACE was on protein breakdown rather than on deamination of amino acids. It was suggested that the availability of sources of

19. Clinical and Metabolic Changes in Addison's Disease Following the Administration of Compound E Acetate (11-dehydro, 17-hydroxy-corticosterone acetate).

P. H. Forsham (by invitation), L. L. Bennett (by invitation), M. Roche (by invitation), R. S. Reiss, A. Slessor (by invitation), E. B. Flink (by invitation) and G. W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

Fourteen patients with Addison's disease were treated with synthetic Compound E acetate, administered as a crystalline suspension in water, supersaturated suspension in oil and pellets. Striking clinical improvement followed the injection of 50 to 100 mg. of Compound E daily for two to six days and was sustained for several months in all 5 patients implanted with five to ten 50-mg. pellets. Absorption averaged 0.5 mg. per pellet per day. The most striking metabolic changes observed consisted of a return towards normal of the electroencephalogram, an increased uptake of radioactive iodine by the thyroid, a transient rise in fasting blood ketone bodies with an increased capacity to tolerate a 24-hour fast, and a small, but definite, retention of sodium and chloride in the absence of supplementary desoxycorticosterone treatment. On desoxycorticosterone a paradoxical loss of sodium and chloride occurred.

A small rise in blood sugar levels was accompanied by a decrease in urinary inorganic phosphorus and creatine excretion. Increase in uric acid excretion, with little or no increase in total nitrogen excretion occurred. Circulating eosinophils were depressed. Urinary 17-ketosteroids increased slightly; 11-oxysteroids did not.

20. Effect of a Single Dose of Desoxycorticosterone Acetate on Electrolyte Metabolism.

Paul Fourman* (by invitation), Edwin J. Kepler,** Edward C. Reifenstein, Jr. and Eleanor F. Dempsey (by invitation).

From the Department of Medicine, Harvard Medical School, the Medical Service of the Massachusetts General Hospital, Boston, Massachusetts; and the Department of Medicine, Mayo Clinic, Rochester, Minnesota.

A normal, 24-year-old, 70 Kg. man was put on a daily intake of 300 ml. of water, 3 Gm. of NaCl, 1 Gm. of KCl and 500 Gm. of glucose, given in 12 two-hourly aliquots. Four experiments were done, two control and two with 30 mg. of desoxycorticosterone acetate (DOCA) injected. Urine was collected in 9 two-hourly and then 4 six-hourly periods and the excretion of K, Na, Cl, P, Ca, N, NH₃ and titratable-acidity-minus-CO₂ was measured. For the 40-hour collection period the DOCA effect was as follows:

Metabolite	Effect	Amount
K	Loss	15.4 ± 3.5 mEq.
Na	Retention	50.5 ± 5.3 mEq.
Cl	Retention	39.2 ± 5.9 mEq.
Ca	Retention	44.9 ± 10.0 mEq.

There was no significant effect on N, P, NH₃, or titratable-acidity-minus-CO₂.

For the positive effects the following relations obtained:

mEq. Na retained

$$= (\text{mEq. Cl retained}) \times 1.00 \pm 0.03 \\ + (\text{mEq. K lost}) \times 0.76 \pm 0.08$$

* Rockefeller Traveling Fellow.

** Deceased.

Attempts were made to determine whether the output of adrenal cortical hormone was mediated by epinephrine and could be prevented by adrenergic block. Injection of dibenamin was found to be an alarming stimulus, leading to an increase in blood levels of cortical hormone. Insulin as well as formalin induced a further rise.

17. Urinary Corticoids.

Eleanor H. Venning, M. P. Ripstein (by invitation) and V. E. Kazmin (by invitation).

From McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

In the following study a comparison of two chemical methods and a biologic assay for urinary corticoids has been made on urine collected from both normal and pathologic. The chemical methods used were based upon 1) the reduction of cuprous ion to cupric ion, and 2) the formation of formaldehyde following periodic acid oxidation. They were carried out on the same urinary fraction, the water soluble neutral corticoid fraction. The biologic assay which is dependent upon the deposition of glycogen in livers of adrenalectomized mice, was done on the total neutral corticoid fraction. The same standard, dehydrocorticosterone, was used for all assays.

In most of the normal cases, the reduction method gave higher values than the oxidation method. Greater variations were encountered with the reduction method than with the oxidation method.

In 9 cases, in which the biologic assay was below the normal range, with the exception of 2 cases, the values obtained by the chemical methods were also low. These patients suffered from pituitary tumors, Addison's disease, myxedema and malnutrition.

In 5 different lots of pooled urine collected postoperatively from patients, there was considerable disagreement between the biologic and chemical assay.

A study was also made on the effect of pH, and time, on the hydrolysis of urinary corticoid conjugates in urine.

18. Studies on the Interrelationship of Adrenal and Thyroid Function.

Robert S. Reiss, Peter H. Forsham (by invitation) and George W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

In 8 patients with Addison's disease the somewhat low gradients of radioactive iodine uptake, as measured by the method of Astwood and Stanley, following the oral administration of 100 mc. of I^{131} , were increased up to fourfold following the intramuscular injection of synthetic Compound E acetate (50 to 100 mg. per day) and Lipoadrenal Cortex (4 to 6 cc. per day). This effect was maximal after two to three days and decreased thereafter. No such effects were observed in a case of panhypopituitarism.

The simultaneous administration of 0.3 mg. of epinephrine and I^{131} to 5 normal subjects led to an increased rate of iodine uptake by the thyroid, maximal after three to four hours coincidentally with the greatest depression in circulating eosinophils, known to be an indicator of adrenal cortical hormone secretion. Both responses were absent in a patient with panhypopituitarism who did show a positive response to thyrotropin and adrenocorticotrophic hormone. Patients with Addison's disease failed to show either thyroid or adrenal cortical activation following 0.3 mg. of epinephrine subcutaneously.

It is suggested that certain adrenal cortical hormones facilitate the release and/or action of thyrotropin.

with Addison's disease showed little or no change in these constituents. It is concluded therefore that the changes which follow ACTH administration may serve as criteria for the evaluation of adrenocortical reserve.

Ten patients with pituitary tumors were similarly studied. Their response to ACTH was variable. In general they fell into two groups: those whose response was normal, and those whose response was abnormal by one or more of the criteria employed. The effect of the removal of the tumor on the response to ACTH in 4 patients is presented.

23. The Level of Circulating Eosinophils as an Indicator of Adrenal Cortical Adequacy Following Major Surgery.

Marcel Roche (by invitation), A. Gorman Hills (by invitation) and George W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

Earlier studies demonstrated the depression of circulating eosinophils following an increase in adrenal activity. In 43 patients, following major surgery, the level of eosinophils fell from normal (100 to 200/cu.mm.) to, or near, zero in all except 1 patient five to fifteen hours postoperatively. In this patient, fifteen hours following a colostomy for malignancy, the level of eosinophils was 296. At autopsy unsuspected bilateral adrenal tuberculosis was demonstrated.

Following operation, the eosinophils returned to or above preoperative levels within three to five days in most instances. At this time a fall in eosinophil level, in response to the administration of 25 mg. of standard adrenocorticotrophic hormone intramuscularly, or 0.3 mg. of epinephrine subcutaneously, suggests that the postoperative rise in eosinophils is associated with a restoration of normal adrenal cortical reserve. It is suggested that, in patients with complicated postoperative convalescence, adrenal cortical insufficiency may be ruled out by finding an initial eosinopenia or observing a satisfactory response to ACTH or epinephrine.

24. Is the Protein Metabolic Abnormality of Cushing's Syndrome Catabolic or Anti-Anabolic?*

Sheldon Margen** (by invitation), Laurance W. Kinsell, Erin K. Flanagan (by invitation), Lila E. Suiter (by invitation) and Elliot Rapaport (by invitation), with the technical assistance of Vernon T. Thompson.

From the Division of Medicine, University of California Medical School and the Metabolic Research Unit, U. S. Naval Hospital-University of California Medical School, Oakland and San Francisco, California.

S³⁵-labeled-methionine in tracer dosage was administered intravenously to a 39-year-old female with active Cushing's disease, referable to a large tumor of the left adrenal gland. The incorporation of S³⁵ in plasma protein, tissue protein, total urinary sulfate and urinary organic sulfur was determined during the period of study.

Results show that the initial incorporation of S³⁵ into the plasma proteins occurs at a more rapid rate than in the normal. In the first 48 hours there is no greater urinary excretion of organic S³⁵ (S³⁵-labeled-methionine) or S³⁵O₄ (catabolized labeled methio-

* This work was supported by grants from the Research Division, Bureau of Medicine and Surgery, U. S. Navy (BuMed #007046), and from the Office of Naval Research under a contract between the latter and the University of California.

** Senior Research Fellow, U. S. Public Health Service, 1947-48; and Schering Research Fellow in Endocrinology, 1948-49.

mEq. Ca retained

$$\begin{aligned} &= (\text{mEq. Cl retained}) \times 0.74 \pm 0.15 \\ &+ (\text{mEq. K lost}) \quad \times 1.19 \pm 0.41 \end{aligned}$$

Sodium retention was limited by the amount available, and the portion of retained sodium that accompanied chloride was less than the amount of sodium normally found with chloride in extracellular fluid. Some sodium entered the cells, replacing potassium but less sodium entered than potassium left. Calcium retention was correlated with potassium loss independently of calcium retention due to increase of chloride space.

21. Changes in Urinary Steroids Produced by Sodium Deprivation and by Desoxycorticosterone Acetate Administration.

William H. Daughaday (by invitation) and Cyril M. MacBryde.

From the Department of Internal Medicine, Washington University Medical School, St. Louis, Mo.

If sodium reabsorption by the renal tubule is under intimate regulation by the adrenal cortex, it should be possible to detect changes in the excretion of adrenal metabolites under conditions of excess or deprivation of dietary sodium. Daily determinations of the urinary excretion of "cortin," (measured by liberation of formaldehyde from steroid residues, *J. Clin. Endocrinol.*, 8: 166, 1948) and of 17-ketosteroids were done in 3 case studies over periods from one to two months with varied sodium intake and during the daily intramuscular administration of 10 and later 15 mg. of desoxycorticosterone acetate (DCA). Changes in steroid excretion were considered in relation to measured alterations in electrolyte and water balance. A woman with Cushing's syndrome showed elevated excretion of cortin and 17-ketosteroids with explosive and cyclic changes in the levels independent of sodium intake. Decreased excretion of 17-ketosteroids but not of cortin occurred during DCA administration. In 2 women with hypertension but without significant renal disease extreme sodium restriction did not result in increased urinary cortin. During DCA administration cortin excretion increased in one case, but was unchanged in the other.

Conclusion: Release of adrenocortical "salt hormone" is apparently independent of ACTH and 11-oxysteroids, and in these doses DCA does not inhibit ACTH liberation and cortin excretion.

22. The Evaluation of Adrenocortical Function by Ascertaining the Response to a Single Injection of Adrenocorticotropin.

H. W. McIntosh (by invitation), B. Singer (by invitation) and M. M. Hoffman.

From McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Normal individuals, patients with Addison's disease and patients with pituitary tumors were studied in the following manner: control urine was collected from 12 midnight to 8 a.m. At this time an amount of ACTH equivalent to 21 mg. of the Armour standard was injected intramuscularly and urine collected during the subsequent six hours. The concentrations of uric acid, potassium, chloride, 17-ketosteroids and neutral reducing substances present in the control and post-injection urines were determined and the results expressed in relation to the creatinine concentration. The number of circulating eosinophils was determined before and four hours after the injection of ACTH.

In the 10 normal subjects there was, following the administration of ACTH, an increase in each of the urinary constituents studied of from 50 to 350%. The 5 patients

certain females with Cushing's syndrome; thus it presumably can inhibit production of ACTH by the pituitary.

The data support the conclusion that testosterone has both a direct anabolic effect on tissue and an inhibitory effect on the pituitary in Cushing's syndrome.

SATURDAY, JUNE 4, 1949

9:00 A.M.—E. A. DOISY, PRESIDING

27. Metabolism and Distribution of Thiourea in the Rat as Measured with Radioactive Sulfur.

John Schulman, Jr. and Richard P. Keating (introduced by Rulon W. Rawson).

From the Los Alamos Scientific Laboratory, Los Alamos, New Mexico.

Thiourea labelled with S^{35} was injected intraperitoneally into young albino rats. The excretion and tissue distribution were measured at 6, 24 and 48 hours. Three animals were used at each time interval.

More than 98% of the radioactivity was excreted in the urine within 48 hours. Filter paper chromatography indicated that most of the thiourea was excreted unchanged. Chemical analysis showed that contrary to earlier reports 6% was excreted as inorganic sulfate and 6% as ethereal sulfate. Fecal excretion was slight. Traces of radioactive sulfur were found in the expired air.

The highest concentration of radioactive sulfur was found in the thyroid gland, reaching a maximum of 3.5% of the injected dose per gram of thyroid at 24 hours. The ratio of the concentration in the thyroid to the average concentration in the body rose from 11:1 at 6 hours to 37:1 at 24 hours and to 90:1 at 48 hours.

This rising ratio suggests that thiourea is bound in the thyroid gland.

28. The Tracer Technique with Radioiodine I^{131} as a Potential Substitute for the Basal Metabolic Rate Determination in Routine Clinical Practice.

Sidney C. Werner, Lawrence D. Goodwin and Edith H. Quimby.

From the Departments of Medicine and Radiology, College of Physicians and Surgeons, Columbia University and The Presbyterian Hospital in the City of New York.

A simple test to replace the basal metabolism has long been desired, in view of the inherent inaccuracies in the method and the effect of subjective influences upon the result. Accordingly, a large scale experiment has been instituted at the Presbyterian Hospital to compare the uptake of radioiodine with the basal metabolic rate in all ward patients on whom a determination of the latter has been requested.

The results in about 200 patients have been summarized: first, the number of patients in whom the basal rate determination could not be done or was apparently unreliable, and the number who could not be tested with radioiodine because of immediately preceding iodide or thyroid ingestion, etc.; second, the accuracy of the two procedures as a reflection of the final diagnosis; third, cost, speed of testing, technician capacity, and incidence of unexpected difficulties.

It is suggested that consideration should be given to the replacement of the basal metabolism as a screening procedure for thyroid dysfunction. The basal metabolic rate would be determined only if an abnormal tracer uptake were found, when the tracer value appeared discrepant, or when the course after treatment was being followed.

nine) than in the normal. These data therefore demonstrate that there is no primary anabolic defect in Cushing's syndrome; in fact there would appear to be a hyperanabolism which does not, however, compensate for the excessive catabolism that must occur. Incorporation of S^{35} into the protein of tissues obtained at the time of operation and autopsy is discussed.

25. Hypokalemic Alkalosis in Cushing's Syndrome. Observations on the Effects of Potassium Chloride and Testosterone Propionate Therapy.

Robert Teabeaut (by invitation), Frank L. Engel and Haywood M. Taylor (by invitation).

From the Departments of Medicine and Biochemistry, Duke University School of Medicine, Durham, N. C.

A 34-year-old man with Cushing's syndrome due to adrenal cortical carcinoma who showed striking electrolyte abnormalities (serum Na-143.5 milliequivalents/liter, K-2.56 milliequivalents/liter, Cl-83.3 milliequivalents/liter, and CO_2 -41.0 milliequivalents/liter) was studied. He had diabetes mellitus (requiring insulin), hypertension, osteoporosis with collapsed vertebrae, and excreted 7.7 mg. of reducing steroids per day. The electrocardiogram showed characteristic changes of hypokalemia. Intravenous administration of 28 grams of KCl (12.9 milliequivalents K^+ /Kg./hour) restored the serum electrolyte pattern and electrocardiogram to normal in four hours. Twenty-one grams of KCl were retained during an eight hour period. Twenty-four hours later the serum potassium declined to 3.72 milliequivalents/liter, the CO_2 now being 31.5 milliequivalents/liter. In nine days the serum electrolytes had returned to their original levels. One hundred mg. of testosterone propionate daily for nine days resulted in a decline in the serum CO_2 from 37.8 to 21.6 milliequivalents/liter, serum potassium remaining low, *i.e.*, 3.0 milliequivalents/liter. These findings are consistent with the views of Darrow and Kepler that the alkalosis and hypokalemia in this syndrome are secondary to intracellular potassium depletion. Testosterone presumably overcame the alkalosis by promoting intracellular potassium retention at the expense of extracellular potassium, the serum potassium thus remaining low.

26. The Mechanism of Action of Testosterone in the Therapy of Cushing's Syndrome.

Frederic C. Bartter, Anne P. Forbes, William M. Jefferies, Evelyn L. Carroll (by invitation) and Fuller Albright.

From the Massachusetts General Hospital, Boston, Massachusetts.

In previous reports it has been demonstrated that methyl testosterone and testosterone propionate have a beneficial effect in females with Cushing's syndrome. This involves: 1) a marked anabolism of protoplasm; 2) a decrease in 17-ketosteroids of endogenous origin; 3) a decrease in "11 oxysteroid" excretion.

A priori, testosterone could exert its effect by: a) inhibiting or neutralizing the action of adrenal cortical hormones on tissues, b) inhibiting the action of ACTH on the adrenal cortex, or c) inhibiting ACTH production.

Metabolic studies are presented to support the following arguments:

Testosterone produces anabolism of protoplasm in Addison's disease; thus, it must have an effect peripheral to the adrenal cortex.

Methyl testosterone, when administered simultaneously with ACTH, does not exert its full anabolic effect, nor prevent the rise in "11 oxysteroids," the fall in eosinophils, or the rise in 17-ketosteroids; thus it presumably does not inhibit the action of ACTH on the adrenal cortex.

Methyl testosterone does produce a fall in "11 oxysteroids" and in 17-ketosteroids in

weighted shavings and emphasize the faster growth of large than of small hairs and the greater accuracy obtained with more refined measures than the gross weight.

Wide fluctuations occur at different periods of the year, even in the orchiectomized man injected daily with an unvarying amount of testosterone propionate:

32. The Effects of Testosterone Propionate on the Peripheral Blood and Bone Marrow of Women with Advanced Inoperable Carcinoma of the Breast. Preliminary Report.
Timothy R. Talbot, Jr.* (by invitation) and George C. Escher.*

Ten out of 70 patients under treatment with testosterone propionate for inoperable carcinoma of the breast showed an increase in hemoglobin, hematocrit and erythrocyte count: average values 49 to 58%; RBC 5.3 to 6.8 million per cu. mm. Six out of these 10 patients exhibited a moderate increase in cellularity of bone marrow or an increase of the erythroid-myeloid (E/M) ratio. Two of these patients had pulmonary metastases although neither showed an increased cellularity of the bone marrow or an increased E/M ratio.

No radiotherapy was given within a six month period prior to or during hormone therapy.

These data are offered as confirmatory evidence in the human female of changes that have been previously observed by others in animals. No explanation of this action of testosterone propionate can be suggested at this time.

33. Effects of Small Doses of Testosterone Propionate on Spermatogenesis.
Cleve Beller† (by invitation) and Henry H. Turner.

From the University of Oklahoma School of Medicine and Oklahoma Medical Research Foundation, Oklahoma City.

Studies are in progress to determine the effects of 5 mg. of testosterone propionate given parenterally twice weekly to subjects showing aspermia, oligospermia and normal sperm counts.

Subjects consists of a number of healthy medical students, and the series is being controlled by administration of sesame oil to a similar group. To date the normal subjects have shown no significant deviation in sperm count. The majority of those individuals with oligospermia are showing a significant increase in sperm count, while the single case of aspermia has shown no change.

The study is being extended to similar groups noting response to 2.5 and 1.25 mg. of testosterone propionate twice weekly for eight weeks.

34. Endocrine Factors in Gout: The Significance of Differences in Childhood and Adult Urate Metabolism.

William Q. Wolfson, David Krevsky, Rachmiel Levine (by invitation), Kinu Kadota (by invitation) and Clarence Cohn.

From the Departments of Biochemistry, ‡ Metabolic and Endocrine Research, and Pediatric Research, Medical Research Institute, Michael Reese Hospital, Chicago 16, Illinois.

Children's urate metabolism has been found to differ from the normal adult's in a

* Assistant, Sloan-Kettering Institute, Memorial Cancer Center, New York; Fellow American Cancer Society sponsored by the Committee on Growth of the National Research Council.

† Schering Research Fellow in Endocrinology 1948-1949.

‡ Aided by a grant from the Committee on Scientific Research of the American Medical Association.

29. The Distribution and Metabolism of Circulating Testosterone.

C. D. West and L. T. Samuels.

From the Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City 1, Utah.

Testosterone in solution in plasma was administered intravenously to rabbits, dogs and men. The rate of disappearance from the blood stream was determined, both in the intact individual and, in animals with the liver, kidney, or both liver and kidneys eliminated from the circulation. Elimination of either organ reduced the rate of disappearance while the elimination of both further reduced, but did not eliminate, the disappearance of the hormone.

Testosterone accumulated in greatest concentration in the fatty tissues, and next in the spleen, heart and skeletal muscle. All of these tissues had concentrations as high or higher than the blood at the time of sampling. Other tissues had lower levels; liver contained no significant amount.

In man, the 17-ketosteroid excretion curve was determined in relation to the blood testosterone and 17-ketosteroid levels. No significant amount of testosterone appeared in the urine even when blood levels were high. The effect on nitrogen metabolism is also discussed.

30. Pseudo-hypoparathyroidism: a Report of Two New Cases With Special Reference to the Epiphyseal Changes.

Harold Elrick (by invitation), Frederic C. Bartter, Adney Sutphin (by invitation) and Fuller Albright.

From the Massachusetts General Hospital, Boston, Massachusetts.

In 1942 (*Endocrinology* 30: 922-932) the syndrome of pseudo-hypoparathyroidism was described. This syndrome resembles hypoparathyroidism with respect to the disorder of calcium and phosphorus metabolism but the immediate cause of the disorder is not a lack of hormone but a failure to respond to it. In addition, it is often associated with a characteristic facies and certain epiphyseal changes in the hands and feet.

Two new cases are reported. In one case x-rays from ages three to seventeen are available and demonstrate the evolution of the unusual epiphyseal changes. Parathyroid biopsy in one of the cases confirmed the presence of normal or hyperplastic parathyroid tissue.

Attention is called to a new physical sign characterized by peculiar movement of the lip during speaking.

31. Quantitative Measurements of the Growth of Axillary Hair as an Index of the Endocrine Status.

James B. Hamilton.

From the Department of Anatomy, Long Island College of Medicine, New York.

A continuously-growing secondary sex character, axillary hair, can be measured quantitatively. In 45 apparently normal men, 21 to 75 years of age, the curve of average values per decade of life parallels that for urinary steroids, although for individual men the parallel is far from exact. After the third decade of life the average curve of decline in weight of axillary hair in 420 subjects is straight in men. In women it is sigma-shaped and falls much more rapidly beginning with the fifth decade. The values are low in eunuchs, and are nil or less than 1 mg. in prepubertal castrates.

Weekly measurements of separately identified hairs confirm the findings obtained with

Because of the specificity of this enzyme, it follows that the sulfate esters of estrone, estradiol and estriol hydrolyzed by the enzyme were conjugated at the phenolic hydroxyl at carbon 3.

37. Hormonal Factors Producing the Gametokinetic Response in the Male Frog (*Rana pipiens*).

Robert B. Greenblatt, Sarah Clark (by invitation) and R. M. West (by invitation).

From the University of Georgia School of Medicine, Augusta, Georgia.

Various commercial pituitary gonadotropins, prolactin, chorionic gonadotropin, equine gonadotropin and pregnancy urine have been found to produce the gametokinetic response in the adult male frog (*Rana pipiens*) and the minimal dose necessary to give this response has been determined.

The minimal dose of gonadotropin necessary to produce the gametokinetic response in the *Rana pipiens* frog has been compared with the minimal amount of gonadotropins necessary to produce ovarian hyperemia in immature Sprague-Dawley rats. In general, smaller amounts of the gonadotropins will give a positive rat hyperemia test than are necessary for the release of sperm in the male frog (*Rana pipiens*).

The male frog (*Rana pipiens*) has proved to be a reliable test animal for the diagnosis of pregnancy. In our series of 111 cases there have been 5 false negative tests. In all cases the rat ovarian hyperemia test and the *Rana pipiens* test were employed. No false positive frog tests were encountered. The rat hyperemia test was positive in the 5 cases in which discrepancy was noted.

38. Action of Estrogens on Release of Luteinizing Hormone in Menopausal Women.

Arthur A. Hellbaum, J. W. Funnell (by invitation) and E. C. Keaty (by invitation).

From the University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Changes in the amounts of luteinizing (LH) and follicle stimulating (FSH) hormones in the urine from women in the menopause were determined before, during, and after the administration of various estrogens. Ovarian changes in normal and hypophysectomized immature rats were used for the assay of the gonad stimulating factors.

With large doses of estrogenic preparations it was possible to demonstrate the presence of LH in the urine of patients in whom prior to treatment only FSH had been demonstrable. The increased presence of LH in the urine was correlated with symptomatic improvement. Estradiol esters were more effective than stilbene derivatives in releasing the LH from the pituitary.

39. The Hormonal Pattern in Pseudocyesis.

A. E. Rakoff and Paul H. Fried (by invitation).

From the Jefferson Medical College and Hospital, Philadelphia, Pa.

This study included 24 women presenting the typical clinical manifestations of pseudocyesis, including amenorrhea or hypomenorrhea, abdominal enlargement, weight gain, enlargement of breasts with galactorrhea, plus a strong belief that they were pregnant and felt fetal movement. Urine hormone assays consistently showed absent or low titers of gonadotropins and high titers of estrogens. Endometrial biopsies in 10 patients showed evidence of progesteronic activity in 9 cases, with a late secretory endometrium in

direction generally opposite to the deviations in gout. Children's average plasma urate levels (3.3 mg.%) are lower even than normal women's (4.2 mg.%) and show no sex difference. Urine ratios of urate/preformed creatinine (0.68) and urate/total creatinine (0.52) and of urate clearance/endogenous creatinine clearance (0.15) are all higher in children than in adults. Others have ascribed the latter findings to relatively increased corticoid activity. Our data are consistent with the known high corticoid/androgen ratio in childhood. Cushing's syndrome, and sometimes acromegaly, show high corticoid/androgen ratios, and may show a childhood type of urate metabolism.

Androgenic regulation of urate metabolism is suggested by higher average plasma urate levels in men than women, both in normals and the gouty. Pre-gouty carriers of hereditary hyperuricemia have normal urate levels until puberty, if male, and until the menopause, if female (Smyth, Stecher and Wolfson: *Science* 108: 514, 1948). Such observations imply androgenic control of hereditary hyperuricemia. A separate abnormal androgen has been postulated because a sex differential in plasma urate exists in gout. Very low 17-ketosteroid excretion in gout without hypogonadism and with unimpaired ability to convert administered testosterone to 17-ketosteroid also suggests an abnormal androgen (*J. Clin. Endocrinol.* 9: 497 (June) 1949).

35. The Effect of Castration, of Unilateral Castration, and of Pregnancy in Unilaterally Castrate Rats on the Ovary Transplanted into the Spleen.

Gerson R. Biskind and Morton S. Biskind.

San Francisco and New York.

A regular sequence of events occurs after one ovary has been transplanted into the spleen of a castrate rat. After the initial inflammatory reaction has subsided there is a constant development of new follicles which luteinize. These corpora lutea do not involute and from them, after five months or more, a luteoma appears. Subsequently a granulosa cell tumor may replace the luteoma. If an ovary is transplanted into the spleen of a unilaterally castrate rat, the intrasplenic ovary atrophies. The growth potentialities of this atrophic intrasplenic ovary become evident as soon as the normal ovary is excised. The effect of pregnancy on the intrasplenic ovary of unilateral castrates is to produce active follicular growth without the formation of corpora lutea. Following termination of the pregnancy the intrasplenic ovary atrophies; however simultaneous termination of the pregnancy and castration produces active growth in the intrasplenic ovary. Certain aspects of the unusual hormonal stimulation in these experimental states are discussed.

36. The Occurrence of Conjugated Sulfates of Estrogens in Human Pregnancy Urine.

Herman Cohen (by invitation) and Robert W. Bates.

From the Endocrine Development Department, E. R. Squibb and Sons, New Brunswick, New Jersey.

The conjugation of estrogens in human urine with both sulfuric and glucuronic acid has been postulated, but no sulfate conjugated estrogen has been demonstrated.

Hydrolysis with the phenolsulfatase present in commercial enzyme preparations obtained from *aspergillus oryzae* was carried out directly on urine or on aqueous solutions prepared from butanol extracts from urine. It was demonstrated that a variable portion of the estrogens in human pregnancy urine is excreted as the sulfate ester. Among 7 different urine samples obtained from women in the seventh to ninth month of pregnancy, it was found that 5-89% of the estriol fraction and 8-100% of the estrone-estradiol fraction occurred conjugated with sulfuric acid.

42. Studies in Carbohydrate Metabolism in Decerebrate Rats.

Evelyn Anderson and Webb Haymaker (by invitation).

From the National Institute of Health, Bethesda, Md., and Army Institute of Pathology, Washington, D. C.

It has been demonstrated by Claude Bernard and by numerous other workers since his time that in acute experiments in rabbits and cats, piqure of the floor of the fourth ventricle or decerebration at the pontile level gives rise within less than an hour to hyperglycemia and glycosuria and that the increase in the blood sugar level is usually maintained for three or four hours, but may last as long as nine hours. In all of these experiments the animals generally survived not more than a day or two.

It is the purpose of this communication to describe the effects of decerebration upon the carbohydrate metabolism of rats which survived the operation for one to three weeks. Decerebration was done at pontile and midbrain levels. Glucose tolerance tests, insulin sensitivity and liver and muscle glycogen determinations were done on the rats three to seven days after decerebration. There was a marked elevation of the glucose tolerance curve during this postoperative period. The insulin sensitivity and the levels of glycogen in liver and muscle appeared to be normal.

43. Factors Affecting the Volume of the Islands of Langerhans.

R. E. Haist, Margaret Evans and B. Kinash (introduced by C. H. Best).

Abstract not presented.

44. Studies on the Serum Potassium in Diabetic Acidosis.

Carl S. Nadler, Samuel Bellett and Mary Lanning (introduced by C. H. Best).

Abstract not presented.

45. Pyruvic and Citric Acid Metabolism.

Max Miller and Ernest Bueding (introduced by C. H. Best).

Abstract not presented.

46. Changes in Inorganic Serum Phosphorus During the Intravenous Glucose Tolerance Test as an Adjunct to the Diagnosis of Early Diabetes Mellitus.

Peter H. Forsham (by invitation), Marcel Roche (by invitation) and G. W. Thorn.

Abstract not presented.

47. The Metabolism of Glucose and Galactose when Administered Simultaneously to Man.

G. C. Walsh (by invitation), M. M. Hoffman, H. T. McAlpine (by invitation) and E. H. Mason (by invitation).

From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

A method which might be helpful in the elucidation of the pathogenesis of hyperglycemia in man is presented. A solution containing 25 Gm. of glucose and 25 Gm. of galactose is administered intravenously to subjects in the postabsorptive state. From specimens of venous blood the glucose and galactose concentrations at 15, 30, 45, 60 and 120 minutes following the injection are determined. The rate of removal of each sugar from the blood stream is expressed as a Removal Constant which is calculated from the concentrations of that sugar at 15 and 45 minutes. The results obtained in normal subjects and patients are shown in the following table.

2, early secretory endometrium in 2 and a mixed type of endometrium in 5. It is postulated that these patients have a persistence of corpus luteum functions, probably maintained by luteotropic hormone (prolactin). Preliminary studies indicate the presence of the latter in the urine extracts of 4 patients, tested by the method of local application to the crop sac of the pigeon. This mechanism adequately explains most of the clinical features observed. Psychiatric interviews suggest that the alteration in endocrine function has its origin in a profound psychic disturbance.

40. Management of Threatened Abortion in the Human with Large Doses of Diethylstilbestrol.

A. B. Abarbanel.

From the Department of Obstetrics and Gynecology and the Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

In a consecutive series of 100 cases of threatened abortion lactose placebos were alternated with diethylstilbestrol.

There were 50 cases of threatened abortion occurring before day 70 (from first day of last regular menses). With placebos, the salvage rate in the control group was 20%. With diethylstilbestrol the salvage rate was 35%.

In the 50 cases occurring between day 70 and 120, the salvage rate was 40% for controls and 84% for those treated with diethylstilbestrol.

It is postulated that 1) the poor salvage rate before day 70 of gestation is due to the fact that most of these cases are caused by blighted ova, or disturbances in the trophoblast, as well as a break in the vascular continuity of the endometrial vascular bed; 2) after day 70 of gestation, when organogenesis is more complete, the most common cause of threatened abortion is a break in the placental-endometrial vascular bed. Since estrogen is a prime factor in maintaining vascular continuity of the endometrial vessels, it is felt that large doses of estrogen will repair the break in the dike, so to speak, and thus the pregnancy will tend to be salvaged.

Joint Meeting with The American Diabetes Association

2:00 P.M.—C. H. BEST, PRESIDING

41. A Hyperglycemic Factor Extracted from the Pancreas.

I. J. Pincus (introduced by A. E. Rakoff).

From the Department of Physiology, Jefferson Medical College, Philadelphia, Pa.

Studies have been performed on a hyperglycemic factor obtained from the pancreas. This substance can be extracted from normal dog pancreas, from pancreas rendered fibrotic by ligation of the pancreatic ducts, pancreas obtained from animals previously treated with alloxan, and fetal calf pancreas before the acinar tissue is secretory. All brands of insulin tested have also shown the presence of this activity, best demonstrated when American insulins have been treated so as to destroy their hypoglycemic action.

Administration of this factor in dosage sufficient to elevate blood sugar levels significantly had no effect on blood pressure. It was also ineffective in stimulating the external secretory activity of the pancreas.

It is possible to demonstrate the ability of this factor, when administered intravenously, to prevent hypoglycemia produced by small doses of insulin. However, when this substance has been administered after large amounts of insulin, we have not succeeded in preventing a fatal outcome in a significant number of animals.

Further studies are being carried out on the possible physiologic significance of this substance.

sibilities is considered, as well as the evidence which might indicate an inhibiting effect of testosterone upon the production of agents such as adrenocorticotropin, the net effect of which could be the production of excessive ketogenesis.

Additional studies in other individuals relating to the effect of testosterone upon infused sodium aceto acetate are also presented.

49. The Urinary Excretion of Corticosteroids in Diabetic Acidosis.

Janet W. McArthur, Randall G. Sprague and Harold L. Mason.

From the Massachusetts General Hospital, Boston, Mass., and the Mayo Clinic, Rochester, Minnesota.

The urinary excretion of corticosteroids has been measured chemically in 6 patients with diabetes mellitus during the first 24 hours after their admission to the hospital in diabetic acidosis, and during a 24-hour "control" period after their recovery. It was found that the rate of urinary excretion of corticosteroids during the period of acidosis was from two to eight times as rapid as during the control period. There appeared to be a direct correlation between the severity of the acidosis, as judged by the level of CO_2 combining power on admission, and the rate of excretion of corticosteroids.

The temporal relation between clinical manifestations of diabetic acidosis and laboratory indications of increased adrenal cortical activity was investigated by withdrawing insulin from a diabetic patient under controlled conditions. Adrenal hyperactivity could be detected by a fall in circulating blood eosinophils within 24 hours after the withdrawal of insulin. However, an increase in the rate of excretion of corticosteroids could not be demonstrated until mild acidosis was clinically apparent.

50. "Steroid Diabetes" Associated with Cushing's Syndrome and Excretion of 17-Hydroxycorticosterone (Compound F) in Urine; Metabolic Studies.

Randall G. Sprague, Alvin B. Hayles (by invitation), Harold L. Mason, Marschelle H. Power (by invitation) and Warren A. Bennett (by invitation).

From the Mayo Clinic, Rochester, Minnesota.

Observations were made in the case of a 14-year-old boy with severe diabetes associated with Cushing's syndrome. The urinary excretion of "11-oxysteroids" was remarkably high (about 17 mg. daily, or about 20 times normal). This finding led to the isolation of 17-hydroxycorticosterone (compound F), 191 mg. of purified hormone being obtained from a twenty-five-day collection of urine.† The diabetes exhibited the features of "steroid diabetes" in animals. It was severe, glycosuria being incompletely controlled with 130 units of insulin daily. Unlike the usual behavior in ordinary juvenile diabetes, however, the urine became virtually free of sugar (2.4 Gm. in twenty-four hours) and ketonuria was absent when food and insulin were withheld for twenty-four hours. Nitrogen balance was markedly negative with an intake of 57 Gm. of protein daily. As suggested by the presence of osteoporosis, the balances for calcium and phosphorus were also negative.

The patient died after resection of one adrenal gland. At necropsy, pronounced hypertrophy and hyperplasia of the adrenal cortices, a small thymoma, a parathyroid adenoma, osteoporosis and bilateral renal calculi were found.

The metabolic behavior outlined, plus the finding of 17-hydroxycorticosterone in the urine, seems to justify the conclusion that the patient had "steroid diabetes" analogous

† Mason, H. L. and Sprague, R. G.: Isolation of 17-hydroxycorticosterone from the urine in a case of Cushing's syndrome associated with severe diabetes mellitus, *J. Biol. Chem.* 175: 451-456 (Aug.) 1948.

Condition of Subjects	Number	Glucose Removal Constant		Galactose Removal Constant	
Normal	33	2.1	(1.0 -4.0)*	6.7	(4.5-9.9)*
Acute hepatitis	12	0.92	(0.42-2.27)	3.1	(2.3-4.0)
Chronic hepatitis	11	0.8	(0.5 -1.3)	2.4	(1.2-3.9)
Diabetes	28	0.57	(0.03-1.52)	6.0	(4.0-8.7)

* Mean and range.

These results indicate that patients with hepatitis who have impairment of glucose tolerance also have impairment of galactose tolerance. Patients with diabetes, on the other hand, show impairment only of glucose tolerance. The possible application of this procedure to the investigation of the causes of hyperglycemia will be discussed.

48. Studies in Fat Metabolism. I. Steroid Hormonal Effects Upon Blood Ketones and Other Intermediate Products of Fat and Protein Catabolism.*

Laurance W. Kinsell, Sheldon Margen** (by invitation), George D. Michaels (by invitation), Betty T. Signorotti (by invitation) and David P. McCallie (by invitation); with the technical assistance of Vernon T. Thompson, Robert V. Deal, and Philip LoDuca.

From the Division of Medicine, University of California Medical School, Metabolic Research Unit, U. S. Naval Hospital-University of California, and the Department of Medicine, U. S. Naval Hospital, Oakland and San Francisco, California.

A patient with severe diabetes mellitus complicated by severe thyrotoxicosis was followed under balance study conditions for a period of more than three months. His diet and insulin were so regulated as to cause him to catabolize more than 260 Gm. of fat daily, to lose approximately 75 Gm. of glucose in the urine each day, and to be in negative nitrogen balance. Under these conditions, his fasting blood ketones were more than 30 times the normal level and he had extensive ketonuria.

With all other factors remaining constant, testosterone propionate administered to this man in a dosage of 150 mg. daily, produced a profound protein anabolic effect, a decrease of glycosuria of proportional degree ($D/N = 3.65$), and at the same time produced a progressive fall in the fasting blood ketones to levels approaching the normal. During this same period, his basal metabolic rate was not significantly changed, and his fat catabolism was therefore as great or greater than during the period prior to testosterone administration. The discontinuance of testosterone for a period of two days resulted in a prompt rebound in the entities above noted as well as the production of other metabolic changes which are discussed.

From the above, one must conclude that under the conditions of this study testosterone produces a profound effect upon intermediate products of fat and protein catabolism and that this effect is most strikingly manifested by a diminution in fasting blood ketones. This observation may represent an acceleration of ketolysis or a diminution in ketogenesis from protein and/or fat. The evidence in favor of each of these pos-

* This work is supported by grants from the Research Division, Bureau of Medicine and Surgery, U. S. Navy (BuMed #007046), and from the Office of Naval Research under a contract between the latter and the University of California.

** Senior Research Fellow, U. S. Public Health Service, 1947-48 and Schering Research Fellow in Endocrinology, 1948-49.

53. The Effect of Pteroylglutamic Acid Antagonists on the Response of the Reproductive Accessories of C57 Male Mice to Testosterone.

E. D. Goldsmith, H. M. Black (by invitation) and R. F. Nigrelli (by invitation).

From the Department of Histology, College of Dentistry, New York University and New York Aquarium, New York Zoological Society, New York.

Preliminary investigations disclosed that the seminal vesicles and coagulating glands of castrated male mice receiving a Purina chow ration supplemented with 10 Gm./kilo of succinylsulfathiazole and a high level of a crude folic acid antagonist (x-methylpteroylglutamic acid, Lederle) responded only slightly to high dosages of testosterone.

Seventy day old mice, castrated at 50 days of age, were placed on 1) *the control ration*—Purina chow supplemented with 10 Gm. of succinylsulfathiazole per kilo, and 2) *the antagonist ration* consisting of the control ration to which a folic acid antagonist of known chemical constitution, 4-amino-N¹⁰-methyl-pteroylglutamic acid (Amethopterin, Lederle) was added at levels of 17 mg. per kilo. After eleven days, the "antagonistic" animals were given daily injections of 1 mg. of testosterone propionate in sesame oil during a 5-day period. A number of "control" mice were treated similarly with the androgen, and an equal number were injected with equivalent quantities of sesame oil. All animals were sacrificed 24 hours after the fifth injection. The seminal vesicles and coagulating glands of the testosterone-treated mice on the control diet exhibited the characteristic hypertrophy resulting from androgen treatment. The sesame oil elicited no visible growth. The mice fed Amethopterin showed a reduced response to the testosterone, and differed but slightly from the control stock animals which received sesame oil. Histologic studies are reported.

54. Synthesis of Testosterone from Androstenedione-3,17 by Testis Tissue.

Leo T. Samuels, Blaine H. Levedahl (by invitation), M. L. Helmreich (by invitation) and M. M. Pottner (by invitation).

From the Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City 1, Utah.

Androstenedione-3,17 has been incubated with testis slices from several species of animals. Evidence from colorimetric reactions and biologic assay indicates that this tissue is able to reduce the ketone group on carbon 17, thus forming testosterone. The metabolism of other steroids by testis tissue has also been studied in an attempt to trace a possible route of synthesis of the hormone from constituents known to be present in considerable amounts in testis tissue.

55. The Role of the Adrenal Cortex in Some Somato-Sexual Aberrations in Infants and Children.

M. M. Melicow.

From the Department of Urology, Columbia University, College of Physicians and Surgeons, New York 32, N. Y.

The article is based on a study of somato-sexual aberrations in infants and children seen at the Squier Urological Clinic and Babies Hospital of Columbia University-Presbyterian Hospital.

In this study are presented 2 true hermaphrodites, 13 pseudohermaphrodites, and 7 children with accelerated puberty characteristic of their own or opposite sex.

It was found that at birth and in early infancy the problem is mainly one of ascertaining the true sex and correcting the deviation. Several females were brought up as males

to that produced in animals by the administration of carbohydrate-active adrenal steroids.

51. Behavior of Electrolytes During Treatment of Diabetic Keto-Acidosis.

Jonas Weissberg (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Dreker.

From The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York, N. Y.

Electrolyte balance was studied in 13 diabetic patients before and after the institution of adequate insulin and fluid therapy. Specimens were collected at intervals from one to three hours for the determination of serum potassium, sodium, chloride and phosphorus, CO₂ combining power, blood sugar and urinary potassium, sodium, chloride, phosphorus and total nitrogen, before and after treatment.

All 13 patients presented a definite fall in levels for potassium in the serum at some time during the period of treatment with insulin. Five patients showed a fall in serum potassium to critically low levels. The behavior of phosphorus paralleled that of potassium; sodium and chloride tended to deviate in an opposite direction to values for potassium.

The importance of the alteration of potassium in the diabetic patient is the fact that marked lowering of the level of potassium may be associated with a definite clinical state termed "potassium-depletion syndrome" (hypopotassemia), of which restlessness, marked muscular weakness and hypotonia, fall in blood pressure, tachycardia and occasionally diaphragmatic paralysis are the most characteristic manifestations.

Among the clinically applicable implications of the results are: 1) a means for recognizing the potassium-depletion syndrome is important to the care of all diabetics; 2) frequent determinations of potassium in the serum are necessary during the period of active treatment for the diabetic-acidotic state; and 3) potassium parenterally should be administered only when the serum level falls to critical or "near-critical" levels.

Read by Title

52. Correlation of Vaginal Smears and Endometrial Biopsies in Normal Cycles and in Gynecic Disorders.

H. E. Nieburgs, Robert B. Greenblatt and S. Bamford (by invitation).

From the University of Georgia School of Medicine, Augusta, Georgia.

In the perfunctory study of patients with gynecic disorders a discrepancy was noted not infrequently in the results of the endometrial biopsy and the vaginal cytologic smear. A close correlation should exist between the endometrial histology and the vaginal cytologic smear since both reflect ovarian function. A special study was therefore undertaken in which endometrial biopsies and vaginal smears were taken at the same time in 250 patients with various endocrine-gynecologic disorders. An evaluation of this correlative study is presented. Of particular interest was the finding of two specific types of smears: the "cytolytic" type which heretofore has been interpreted as indicative of progesterone activity and the "mucoid cornified" type which was encountered in patients with clinical evidence of increased responsiveness to intrinsic androgens. A lack of correlation with the endometrial biopsy existed in this particular group of patients. It is interesting to note that the cytolytic smear was associated in most instances with an estrogenic endometrium or cystic glandular hyperplasia, whereas the mucoid cornified type of smear appeared mainly in patients with cystic glandular hyperplasia.

58. Pregnanediol Excretion in Cases of Blighted Ovum.

A. B. Abarbanel, Robert Hoyt (by invitation) and M. G. Levine (by invitation).

From the Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

Pregnanediol excretion was determined quantitatively by a modification of the Astwood-Talbot method and was measured quantitatively spectrophotometrically.

In a series of infertility patients who conceived, pregnanediol excretion was measured at least once a week. In 4 cases in which a blighted ovum was recovered the pregnanediol excretion fell to or towards zero from a previously normal level from two to four weeks before the blighted ovum was expelled. Diagnosis was confirmed histologically. In two cases, the test for chorionic gonadotropin was found to be positive.

In 17 other cases, a presumptive diagnosis of blighted ovum was made on the basis of a drop in pregnanediol with a positive test for chorionic gonadotropin.

From these findings, it is postulated that a viable more or less normal embryo may be actively concerned with the metabolic conversion of progesterone to pregnanediol in the human.

59. A Mechanism of Potassium Deficiency in Alkalosis.

Charles H. Burnett, Belton A. Burrows (by invitation) and Robert R. Commons (by invitation).

From the Evans Memorial Hospital and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts.

Evidence of intracellular potassium deficit has been demonstrated in a series of 12 patients with alkalosis resulting primarily from loss of gastric contents; some were refractory to treatment until potassium was supplied. The mechanism is not clear, but the authors' observations indicate that renal excretion of potassium, which exceeded that anticipated from protein catabolism alone, was an important feature. At least three factors operated in this renal loss: 1) the failure of normal kidneys completely to conserve potassium, even in the presence of potassium deficit; 2) the even greater inability to conserve potassium in renal insufficiency (which has regularly occurred in this type of alkalosis); 3) increases in potassium excretion after administration of sodium salts and hence accentuation of any previous deficiency of this cation (saline was always administered in cases observed). Estimations of tubular reabsorption of various ions (calculated from inulin clearance) indicated that this last effect may be due to inhibition by sodium of renal tubular potassium reabsorption. Alternatively, since there is some evidence that potassium is actively secreted by renal tubules, sodium may stimulate potassium secretion. It is suggested that this phenomenon may offer a clue to solution of the general problem concerning the mechanism of transfers of sodium and potassium across cell membranes.

60. Effects of Hemopoietic Agents on Blood Formation in Hypophysectomized Rats.

Robert Gerstner (by invitation) and Albert S. Gordon.

From the Department of Biology, Washington Square College of Arts and Science, New York University, New York.

The anemia induced in the rat by hypophysectomy can be effectively prevented or overcome by appropriate hormonal therapy (Gordon and Charipper; Crafts). Attempts were made in the present work to influence blood formation in the hypophysectomized rat by the administration of nonendocrine hemopoietic factors. The combination of folic

and vice versa; in a number, the true sex was in doubt from birth. Awareness of error in some cases was not realized until puberty. At birth and in early infancy, clinical, laboratory and radiologic evidence of cortical hyperfunction is usually not demonstrable. In some it becomes manifest later. Steps necessary to demonstrate cortical hyperfunction \pm neoplasia are presented. In childhood, the problem is mainly one of halting the tendency to apparent sex-reversal and removing the cause. The true sex had been ascertained at birth but, because of a profound heterosexual endocrine drive, a somato-sexual aberration developed. Cortical hyperfunction was demonstrable in all cases.

56. Effect of Compound E on Blood Ketone Bodies.

Leslie L. Bennett (by invitation), Alexander Slessor (by invitation) and George W. Thorn.

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Massachusetts.

Fasting (8 a.m.) blood ketone bodies were determined in 7 patients with Addison's disease before, during and after treatment with 100 mg. daily of synthetic Compound E acetate (11-dehydro-17-hydroxy corticosterone). Control levels varied from 0.34 to 1.27 mg. per cent. In 6 patients a significant rise in blood ketone level occurred following the initial 24-hour period of Compound E administration. The values varied from 1.21 to 2.57 mg. per cent, being higher in each instance than any previous control level for that patient. The values returned to, or toward, control levels during continued therapy. This was associated, however, in the one patient in whom the respiratory quotient was measured, with a persistent reduction in the fasting nonprotein respiratory quotient throughout the period of therapy.

In 4 patients blood ketone and blood sugar levels were followed during the last 12 hours of a 24-hour fast. Blood ketone and blood glucose values were maintained at a higher level during the period of Compound E administration, as compared to control values obtained with prolonged fasting in the untreated state. It is suggested that, in man, Compound E facilitates the breakdown of fat via ketone bodies.

57. The Effect of Dietary Protein on the Ability of the Liver to Inactivate Estradiol in the Rat.

Joseph W. Jailer.

From the Endocrine Section of the Medical Services, and the Chemical Laboratory of The Mount Sinai Hospital, New York.

Biskind has claimed that in vitamin B deficiency the liver loses its ability to inactivate estrogens. It had been previously shown that it was not the lack of components of the B complex per se that was responsible in this mechanism, but the concomitant inanition (Jailer).

Spayed female rats with pellets of estradiol implanted in the spleen were placed on isocaloric vitamin B deficient diets containing 5, 15, and 50% protein. After an average of twenty days, the rats on the 5 and 15% protein diets showed signs of estrus as determined by vaginal smear, while those on a 50% protein diet remained in diestrus. Uterine weights at the termination of the experiment were: 5%-99.0 mg.; 15%-128.0 mg.; and 50%-65.7 mg. Other similarly treated rats were placed on a diet which was the same except for ample vitamin B complex. The food intake however, was restricted to 3 grams per day per rat. After 25 days on this regimen, the 5 and 15% groups showed signs of estrogen activity, with average uterine weights of 121.6 and 90.0 mg. respectively, while the 50% group did not (uterine weights 67.2 mg.).

III. Plasma CO₂ combining power:

Average of 11 normotensive patients: 31.1 mEq./L. Average of 19 severe hypertensive patients: 33.2 mEq./L.
probability = .6.

Even though 42 per cent of the hypertensive patients had a value greater than any one in the normotensive group, the differences observed were not significant.

IV. Plasma CO₂/Cl ratios:

Average in 11 normotensive patients: .307.
Average in 19 severe hypertensive patients: .335.
probability = .4.

Even though 32 per cent of the hypertensive group had ratios higher than any one in the normotensive group, the difference in the group were not significant.

Although it is possible that there might be a slight adrenal hypersecretion in essential hypertension, the above data indicate that a marked adrenal hypersecretion is unlikely.

63. The White Blood Cell Response of Rats to Adrenalectomy, Stress, and Pantothenic Acid.*

Mary E. Dumm, Paul Roth (by invitation), Paul Ovando (by invitation) and Elaine P. Ralli.

From the Laboratories of the Department of Medicine, New York University College of Medicine, New York.

Large doses of calcium pantothenate have been shown to modify the response of the white blood cells of the rat to adrenalectomy and, in intact rats, to stress. The total white blood cells and lymphocytes were counted in tail blood from young rats kept for thirty days on a pantothenate deficient diet. Following adrenalectomy, the total white blood cells and lymphocytes were followed for twenty days in groups of rats receiving from 0 to 4 mg. of pantothenate daily. Large doses of calcium pantothenate delayed and partially suppressed the increase in total white blood cells and lymphocytes which usually follows adrenalectomy. In another series of experiments the total white blood cells and lymphocytes were counted before and at intervals after moderate stress (swimming) in both intact and adrenalectomized rats on diets providing various intakes of pantothenate. No consistent changes in white blood cells were found after stress in any group of adrenalectomized rats. Intact rats on the high (4 mg. per day) pantothenate diet showed a delayed lymphocyte response to stress as compared with rats on a moderate (0.1 mg.) pantothenate intake. The low initial lymphocyte values previously reported in intact pantothenate deficient rats were further depressed following stress.

64. Role of Emotional Stress in the Survival of Adrenalectomized Rats Given Replacement Therapy.

Miguel R. Covian (introduced by Curt Richter).

From the Psychobiological Laboratory, Johns Hopkins Hospital, Baltimore 4, Md.

Previous work done in this laboratory showed that laboratory Norway rats can be adrenalectomized and then maintained almost indefinitely merely by increasing the sodium chloride available to them, but that their adrenalectomized wild Norway counterparts do not survive on salt alone after adrenalectomy, nor are cortical extracts entirely successful in keeping these wild rats alive. In an attempt to find the explanation for this

* This research was aided by a grant from the National Vitamin Foundation.

acid, liver extract, iron and copper evoked marked reticulocytosis but lowered RBC counts and Hb concentrations. RBC fragility values and sedimentation rates were increased by this treatment. Protein hydrolysates caused slight reticulocytosis, decreased RBC numbers, hemoglobin concentrations and fragility values but sedimentation rates were increased. Treatment with the combination of protein hydrolysates, iron, copper, liver extract, folic acid, ascorbic acid, and certain members of the vitamin B complex induced a strongly marked and continued reticulocytosis. RBC numbers rose only slightly. Hb concentrations and RBC fragility values were not altered. RBC sedimentation rates were greatly increased. The results of additional experiments now being conducted with santhopterin and vitamin B₁₂, singly and in combination with other factors, are also reported.

61. The Relative Effectiveness of Desoxycorticosterone Acetate in Oil Solution and in Pellets Diluted with Cholesterol.

Albert Segaloff.

From the Department of Medicine, Tulane University and The Alton Ochsner Medical Foundation of New Orleans, Louisiana.

Groups of 15 to 20 immature male Fisher rats weighing 40 to 60 Gm. were adrenalectomized. At operation either a pellet was implanted or the first injection of desoxycorticosterone acetate (DCA) in sesame oil was made. The pellets were removed, or injections stopped after 28 days.

Twenty controls with pellets containing only cholesterol and 20 controls injected with sesame oil died on the fourth, fifth or sixth day after bilateral adrenalectomy.

Pellets with 100%, 75% and 50% DCA produced essentially normal growth. Animals implanted with 5% DCA pellets all failed to survive the 28-day experimental period. Growth with pellets of intermediate percentages of DCA was proportionate to the percentage of DCA. Of the animals injected with DCA, none receiving 10 γ per day survived the 28-day experimental period. Those receiving 125 γ daily grew at essentially the normal rate. The intermediate levels produced growth curves differing in proportion to the amount of DCA given.

Comparing the curves reveals that growth was essentially the same for a given amount of DCA either in terms of absorption or injection.

62. Adrenal Cortex Activity in Essential Hypertension.

Louis Tobian, Jr. and Harold Joseph (introduced by Carl A. Bunde).

From the Southwestern Medical College, Dallas, Texas.

We have previously shown that the urinary "formaldehydogenic" corticosteroid excretion was normal in essential hypertension.

Four other procedures have been carried out to examine further adrenal cortical activity in hypertensive patients.

I. Plasma corticosteroids:

The purified lipid fraction was analyzed by both the Heard-Sobel and Corcoran-Page-Lowenstein methods for corticosteroids. The 8 hypertensive patients and 7 normotensive patients exhibited similar plasma "corticosteroid" levels.

II. Fasting blood eosinophil concentration:

Average of 46 normotensive patients: 139.9/cu. mm. (S.D. = ± 85.6).

Average of 40 essential hypertensive patients: 107.7/cu.mm. (S.D. = ± 77.7).
probability = .07

A combination of the above mentioned doses of somatotrophic and androgenic hormones causes a daily body weight gain of almost one gram more than that induced by somatotropin alone; and the growth response of the os penis was slightly greater than to androgen alone.

67. The "Thiocyanate Space" and "Iodide Space" in the Thyroid Gland.

J. F. McClendon, William C. Foster (by invitation) and Emerson Reed (by invitation).

From the Department of Physiology and Research Laboratory of Physiology, Hahnemann Medical College, Philadelphia.

In the study of thiocyanate goiter, errors arise in the determination of the "thiocyanate space" and the "iodide space" in the thyroid gland due to formation of ferrous thiocyanate and to enzyme action during the extraction. To avoid the former, the concentration of ferric ions was increased 10 times and the spectrophotometer was used at 5000°A. To avoid the latter the thyroid was frozen, quickly sliced with a razor, crushed in a supercooled diamond mortar and quickly homogenized in a Wisconsin homogenizer. The proteins were dissolved in water and precipitated with trichloroacetic acid. The "iodide space" is larger than the "thiocyanate space." Thiocyanate cannot interfere with the uptake of iodide by the thyroid unless it is in relatively high concentration.

68. A Comparison of the 17-Ketosteroid Excretion of Cases of Cushing's Syndrome Due to Adrenal Tumor With Those Due to Hyperplasia (Hyperfunction).

Anne P. Forbes, Evelyn L. Carroll (by invitation) and Mary L. Wheeler (by invitation).

From the Massachusetts General Hospital, Boston, Mass.

It is recognized that Cushing's syndrome may result from a benign or malignant tumor or from hyperplasia (hyperfunction) of both adrenals. Hyperplasia presumably results from an over-production of pituitary adrenocorticotrophic hormone (ACTH). Since ACTH has been shown to increase the excretion of 17-ketosteroids, their excretion in such cases should be elevated.

Small adenomas may be found in hyperplastic adrenals; however, in cases where one large adenoma is found the rest of the adrenal tissue is usually atrophied. The production of ACTH has presumably been suppressed by hormones from the tumor. Urinary 17-ketosteroids should be low unless the tumor itself produces 17-ketosteroid precursors.

With malignant tumors one would expect inconstant results.

The adrenals have been visualized in 16 cases of Cushing's syndrome at the Massachusetts General Hospital. In 5 cases of benign adenoma the 17-ketosteroid excretion was low (average 4.8 mg. per 24 hours). In 9 cases of hyperplasia it was elevated (average 26 mg. per 24 hours). In 2 cases of carcinoma it was very high.

The findings in cases collected from the literature are in general agreement with the above.

69. Sex Hormones and Staphylococcus Infections.

Manuel Villaverde.

Linea 755—Vedado, Habana, Cuba.

According to the author's experience, sex hormones do not seem to act directly upon staphylococcus infections, but upon the tissue condition itself *i. e.*, they do not act like antiseptics or antibiotics, but like tissue regulators.

difference, 21 wild Norway rats (freshly trapped in the alleys of Baltimore), 13 Alexandrine rats and 23 laboratory rats on a high salt diet were adrenalectomized and had percuten pellets implanted between the shoulder blades. Twenty days after the operation the rats were replaced in pairs in a box with a floor made of metal rods and subjected to repeated electrical shocks during a period of five minutes. This treatment caused the wild rats (both Norway and Alexandrine) to fight each other with great intensity, but the laboratory rats merely sought to escape, jumping and squealing, and only occasionally struck out against each other. The wild rats which were not operated on fought violently but did not die.

The mortality resulting from this treatment was much lower for the laboratory rats than for the wild ones, indicating that the emotional state of captive wild rats is such that therapy sufficient to offset the loss of adrenals in laboratory rats is insufficient to keep the wild rats alive.

65. The Androgenic Activity of New Esters of Testosterone.

A. J. Bergmann and Lloyd C. Miller (introduced by John S. L. Browne).

From the Sterling-Winthrop Research Institute, Rensselaer, New York.

Numerous new esters of testosterone have been assayed on castrated rats using testosterone propionate as the reference standard. The acids from which the esters were formed included alkoxy- and alkylmercaptoalkanoic acids in which the alkanic chain contained 2, 3 or 4 carbon atoms. It was found possible to classify the esters into three groups according to their androgenic activity relative to testosterone propionate. Most of the esters were distinctly less active than the latter, or just equalled it, but six were significantly more potent. Two of this latter group, testosterone ethylmercaptoacetate and testosterone methylmercaptoacetate are especially potent, being 123 and 133% as strong as the propionate when compared on the basis of molar equivalents of testosterone. Assayed by the comb-growth response induced in three-day old chicks, comparable potencies were observed. These esters have been studied extensively enough to justify clinical trial.

66. Effect of Androgen and Growth Hormone on the Rat's Os Penis.

Wm. R. Lyons, Edward Abernathy (by invitation) and Mark Gropper (by invitation).

From the Division of Anatomy and Institute of Experimental Biology, University of California, Berkeley, California.

Rats hypophysectomized and castrated at the age of 26 days show almost complete growth stasis and their accessory sexual structures remain infantile. The proximal cartilaginous cap of the os penis becomes atrophic.

Testosterone propionate (0.1 mg. daily subcutaneously for 21 days from the day of operation, promotes a gain of approximately 1 gram daily in such animals. The penis shows preputial splitting within seven days and grows at a faster rate than in normal controls. Although general skeletal growth is retarded as in uninjected controls which underwent operation, the penile bone continues to grow as in normal rats. Cartilaginous condyles develop proximally and following their proliferation they are replaced by apparently normal ossification.

Pure hypophyseal somatotropin (Li and Evans) similarly administered in daily doses of 1 mg. induces a weight increase of skeletal growth equalling that of normal animals; but the accessory sexual organs including the penis and its ossicle remain atrophic.

The various conditions under which intravenous estrogen was used also included myomata uteri, fibrosis uteri, endometrial polyps, cystic ovaries, irregular shedding of the endometrium, estrogen-deprivation bleeding. In 30 cases, a successful result was obtained in 26, or 87%.

In addition, the "stoss" effect of intravenous estrogen has been utilized in threatened abortion, severe menopausal syndrome, infertility in female associated with scanty cervical mucus, and several other conditions.

72. The Incidence of Cancer in Endocrine Case Histories.

J. K. Fancher and Jean Brooks (by invitation).

Atlanta, Georgia—478 Peachtree Street, N.E.

One thousand endocrine case records were studied and compared with the histories of 1000 cancer patients and 1000 patients without glandular disorder or cancer. These 3000 cases were taken from the files of the Good Samaritan Endocrine Clinic and the Steiner Cancer Clinic of Atlanta. There was a noticeable parallel between the incidence of cancer in the family histories of the endocrine and cancer groups, with a perceptible decrease in the negative group, *i.e.*, endocrine 29.8%, cancer 29.1%, neg. 21.5%. Malignancy was most frequent in the first and second generations of antecedents in cancer histories, whereas it was highest in the second and third generations of endocrine patients. The spread in negative cases was not remarkable. Females, exceeded males in all groupings. The G.U. and G.I. tracts were most frequently involved. Various charts are shown. All histories were carefully checked and only complete ones used. Although a factor of error in such statistical material cannot be dismissed, it is thought that the prevalence of cancer and the widespread use of various hormonal products enhances the importance of such a study.

73. Renal Clearances in Patients with Cirrhosis of the Liver, With and Without Ascites.*

Stephen H. Leslie, Barbara Johnson (by invitation) and Elaine P. Ralli.

From the Laboratories of the Department of Medicine, New York University College of Medicine, New York.

Twenty renal functional measurements done on 11 patients with cirrhosis of the liver, during and in the absence of ascites, included effective renal plasma flow (Cpah), glomerular filtration rate (Cln), and maximal tubular excretory capacity (Tm pah). Eight measurements were done while ascites was present, 6 when no evidence of ascites was present, and 6 were done immediately following the ingestion of 1500 ml. of tap water. In the group with ascites, 5 patients were actively reaccumulating fluid and 2 were undergoing spontaneous diuresis at the time of measurement. The average Cpah in the former group was 371 cc./min., the average Cln 69 cc./min., and the average Tm pah 62 cc./min. The averages in the latter group were: Cpah 704 cc./min., Cln 136 cc./min., and Tm pah 54 cc./min. Administration of water preceding measurements to one of the patients caused no change. In the group without clinical evidence of ascites, the average measurements were Cpah 846 cc./min., Cln 201 cc./min., and Tm pah 84 cc./min. In 4 patients measurements following a water load were: Cpah 643 cc./min., Cln 153 cc./min., and Tm pah 74 cc./min.

* This research was aided by a grant from the U. S. Public Health Service.

Two cases are recorded. The first one proved that testosterone is a substance capable of inducing furunculosis. Boils appeared following a course of 25 mg. of the hormone, thrice a week. This man developed furunculosis on three occasions following courses of testosterone, and once it was so intense as to combine several furuncles into a carbuncle. He had not had acne in his youth; and after the withdrawal of testosterone only occasional and mild furunculosis developed.

The second patient, a woman, showed a very intense axillary adenitis, which broke and discharged pus every month before the menses. This condition lasted for twelve or fourteen months, becoming worse as time elapsed. The use of 20 mg. of progesterone daily, the days before the expected menses, improved her state: only a small ulcer appeared, with little exudation. During the next intermenstrual period testosterone (12.5 and 25 mg.) was given the first two weeks, and progesterone (20 mg.) the rest of the time; and since then (after seven months) no axillary swellings were produced. But, paradoxically, an annoying acne developed, after testosterone treatment.

Brief considerations on chronic cystic mastitis and on the relationship between infections and endocrines are discussed.

70. The Problem of Allergy to Steroid Hormones.

George P. Heckel.

From the Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Observations of Zondek and Bromberg (*J. Obst. & Gynec. Brit. Emp.* 54: 1, 1947) indicate that allergy to steroid hormones may be responsible for certain disorders related to the genital functions. To explore this possibility skin tests with crystalline steroid hormones have been given to 137 patients. Injections of .05 cc. of oil containing .05 mg. of the steroid were given subcutaneously (just under the corium) on the arms. Estradiol, estrone, estriol, desoxycorticosterone, pregnandiol, pregnanolone, progesterone and testosterone were used. Reactions were read in twenty-four hours, positive ones consisting of faint erythema and/or slight induration and tenderness.

Sesame oil was used in all cases and in a number the same hormones were given in both sesame and peanut oil. Many reactions were observed with sesame oil as the vehicle, but relatively few with peanut oil. The controls (.05 cc. of oil alone) remained negative. Many patients were tested more than once and sensitivity of the skin was found to be variable in the same individuals. Hyposensitization was tried in 41 cases. The highest dilution of the hormone in .05 cc. of oil which failed to elicit a reaction was given subcutaneously one to four times weekly. Twenty-two of the 41 patients improved. Symptoms related to the menstrual cycle and climacteric which were relieved included headache, nausea, dizziness, fatigue, depression, ovarian pain and mastodynia.

71. Intravenous Estrogen in Menometrorrhagia in the Human.

A. R. Abarbanel.

From the Department of Obstetrics and Gynecology and Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

Mixed conjugated estrogens from pregnant mare's urine was especially prepared for intravenous use in the human. On the rationale that if the level of estrogen could be raised rapidly to a sufficiently high level, functional uterine bleeding could be easily controlled, varying doses of estrogen were used. It was found that a dose of 10 mg. was sufficient to control functional bleeding in 6-12 hours in most cases.

Roughly $\frac{1}{3}$ of all cases had a hemosedimentation rate over 20 mm. (1 hour, Westergreen), without a close relationship between the degree of obesity and this test. The presence of edema, of intestinal parasitosis and the classification of various kinds of obesity do not throw any light on the problem. The obese should be investigated more thoroughly, as they are more liable to premature death than normal people.

76. The Effects of Vitamin B, Thyroid, and Adrenal Alterations on the Amino Acid Oxidase Activity of Rat Liver and Kidney.

Samuel R. Tipton and Frances M. Colvin (by invitation).

From the Department of Zoology and Entomology, The University of Tennessee, Knoxville.

Albino rats were maintained on diets with deficiencies in all or some of the B vitamins. The *d*-amino acid oxidase of liver and kidney tissue is decreased (at least in activity) in total B deficiency and in riboflavin deficiency but is not affected significantly by thiamin deficiency. Administration of desiccated thyroid powder to animals on normal diet increases the enzyme activity. The increase is less in conditions of B deficiency and may not appear at all in severe riboflavin deficiency when thyroid powder or thyroxine is given. After adrenalectomy the enzyme activity is decreased to a small extent. The decrease is aggravated by all vitamin B deficiency conditions that have been studied. A protein-deficient diet results in a decrease in the oxidase activity but the effect is much less than that resulting from riboflavin deficiency.

77. The Problem of Endemic Goiter in Yunnan Province.

Isidor Greenwald.

From the Department of Chemistry, New York University College of Medicine, New York.

In a recent review (Curtis, G. M. and M. B. Feitman: *J.A.M.A.* 139: 28, 1949), a paper bearing the title given above (Robertson, R. C.: *J. Clin. Endocrinol.* 1: 285, 1941) is cited as authority for the statement "This remarkable incidence of goiter in Yunnan, resultant to the military stranglehold on the areas furnishing the only supply of salt with a high iodine content, is no mere coincidence." The "military stranglehold" is the Japanese occupation of the coastal provinces. Actually, Robertson did *not* claim that goiter in Yunnan was of recent origin and as a matter of fact, it has been common in Yunnan since before 1867 (Thorel, C.: *Note medicales du Voyage d'exploration du Mekong et de Cochinchine*, Paris theses, 1870). Moreover, the available analyses show that the salt from the coastal provinces was not rich, but rather poor, in iodine (Wang, H., and F. W. Cheng: *Jour. Chinese Chem. Soc.* 3: 345, 1935). As McClendon wrote, "sea salt may not be considered an adequate source of iodine," (Iodine and the Incidence of Goiter, Minneapolis, 1939). It is possible that goiter was more prevalent in 1940 than in 1867 but it is evident that the salt supply had nothing to do with its spread.

Cirrhosis of the liver with actively reaccumulating ascites depressed all of the functions measured. Once diuresis began the functions measured returned to normal. When ascites had been effectively controlled, the measurements showed an elevation of renal plasma flow and glomerular filtration rate above the range of normal. Tm pah remained normal.

74. Porphyria Simulating Anorexia Nervosa.

Bernard A. Watson.

From Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.

This case is presented because a diagnosis of anorexia nervosa was made on a patient who, for a number of years exhibited signs and symptoms of porphyria which were not recognized. The patient had progressive fatigue, intermittent moderate to severe abdominal and leg pains, nausea, vomiting, and finally a reluctance to eat. He was 6'3" tall and weighed 87 pounds on admission to the hospital. A résumé of his signs and symptoms over a 6-year period are presented.

Of special interest is the fact that he had an appendectomy because of abdominal pain, and later a nephrectomy because of "bloody urine." His gradual withdrawal from society, his many psychosomatic complaints, together with his reluctance to eat because he felt that most foods caused his abdominal pain, resulted in such a marked state of undernutrition that a diagnosis of anorexia nervosa was made.

A routine blood count, 17-ketosteroids and albumin-globulin ratio were within normal limits. The B.M.R. was minus 14. The urine was a "bloody color" showing marked porphyria.

The cause of porphyria in this case, as well as its management, are discussed. The fact is stressed that before a diagnosis of anorexia nervosa or pituitary cachexia is made, porphyria should be considered. It is relatively easy to diagnose if borne in mind.

75. Hemosedimentation Test in Obesity.

Aulo Pinto Viégas.

Belo Horizonte, Minas, Brazil.

Two hundred and twenty-six cases of obesity (obesity is defined as increase of at least 20% in weight), 179 women and 47 men, from 5 to 65 years of age, are presented. Their checkup included: clinical examination, blood studies, roentgenogram of skull and chest radioscopy. A classification has been made according to the degree of overweight:

Number of cases	Overweight percentage	Hemosedimentation	
		20 mm. and up	Average of each group
1st group 48	1 to 10	16 cases or 33.3%	18.0 mm.
2nd group 68	11 to 20	23 cases or 34.7%	18.2 mm.
3rd group 49	21 to 30	16 cases or 33.4%	18.2 mm.
4th group 29	31 to 40	16 cases or 69.4%	26.2 mm.
5th group 22	41 to 50	15 cases or 68.0%	30.4 mm.
6th group 10	51 and up	3 cases or 30.0%	17.0 mm.

THE SCHERING FELLOWSHIP

The Schering Fellowship for 1949 was given to *Doctor D. Lawrence Wilson*. Doctor Wilson was selected as the first recipient of this Fellowship. He will work under the direction of Doctor George Thorn in the field of metabolic and endocrine diseases.

Doctor Wilson received the degree of Doctor of Medicine at Queen's University in 1944. Following his internship he served for eighteen months as Regimental Medical Officer in the R.C.A.M.C. He then received a Medical Research Fellowship for two years from the National Research Council of Canada, working during this period in the Department of Biochemistry of the University of Toronto. He fulfilled, as Research Fellow, the requirements for the degree of Master of Arts which he received in June 1948.

THE SQUIBB AWARD

The Squibb Award for 1949 was given to *Doctor Herbert M. Evans*. About thirty years ago Professor Evans began his studies on the physiology of reproduction. Some of this earlier work was published with J. A. Long in a monograph entitled "Oestrous Cycle of the Rat," a contribution which was of great importance in the investigation and subsequent isolation of one of the ovarian hormones. During the following three decades many significant contributions to endocrinology were made by Evans and his associates, the most important perhaps being the discovery of the growth hormone and its subsequent purification and isolation.

Other important aspects of endocrinology have been studied by Evans and his associates in the University of California: 1) the corticotropic hormones of the hypophysis; 2) the gonadotropic hormones of the hypophysis, of pregnant mare serum, and of the chorion; 3) the impairment of the vaginal cycle due to vitamin A deficiency; 4) the recognition of the importance in the rat of a nutritional factor, X, for pregnancy: and subsequently, the isolation and characterization of this factor, vitamin E.

Doctor Evans was born in California in 1882. He studied medicine at Johns Hopkins, receiving the M.D. degree in 1908. He remained on the faculty of that institution until 1915 when he was called to the Chair of Anatomy at the University of California. In 1930 he was made Herzstein Professor of Biology and Director of the Institute of Experimental Biology.

Association for the Study of Internal Secretions

Recipients of Awards for 1949

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

Doctor Ernest M. Brown, Jr. was named to receive the Ayerst, McKenna and Harrison Fellowship for 1949. He was born in 1919 and received the degrees of Bachelor of Arts from West Virginia University in 1941 and of Doctor of Medicine from the University of Pennsylvania in 1944. He served as intern and junior resident at the University of Pennsylvania Hospital 1944-46. Since that time, until April 1948, he was a member of the Army Medical Corps. Doctor Brown will work at the George S. Cox Medical Research Institute with Doctor F. D. W. Lukens on lesions of the islands of Langerhans produced by intravascular infusion of glucose, continuing his studies begun under this Fellowship in 1948.

THE CIBA AWARD

The Ciba Award for 1949 was given to *Doctor George Sayers*. Doctor Sayers developed a new and sensitive method for the assay of the adrenocorticotrophic hormone of the anterior pituitary gland. He found that the ascorbic acid and cholesterol content of the adrenal glands varies inversely with the amount of ACTH administered to the test animal. Having established the method, he applied it to the problem of pituitary-adrenal relationships. The interrelation of the pituitary and the adrenal cortex, and the response of this hormonal system to a variety of stimuli are better understood and can be better studied as a result of his investigations.

Doctor Sayers was born in 1914. He received the degree of M.S. in physics from the University of Michigan in 1936 and the Ph.D. degree in physiological chemistry from Yale University in 1943. From 1943 to 1945 he served with the Office of Scientific Research and Development at Yale University and in 1945 became Assistant Professor of Pharmacology at the University of Utah.

School, Boston, he has been an assistant in medicine, 1946-48, and an instructor from 1948 to the present time.

SECOND HONORABLE MENTION

Second Honorable Mention was given to *Dr. Ruth Cortell* of Mt. Sinai Hospital, New York, for her essay on "The Antithyroxine Activity of Thyroxine Analogs."

Dr. Cortell received the B. A. degree from Wellesley College in 1935, the Ph. D. degree in Physiology from the University of Chicago in 1939 and the M. D. degree from Yale University School of Medicine in 1948. She did research work at the University of Illinois School of Medicine, 1939-41, at the Abbott Laboratories, 1941-42, in the Thyroid Clinic of the Massachusetts General Hospital, Boston, 1942-44, and then entered Yale Medical School, graduating in 1948. During this entire period she was the recipient of the Smith, Kline & French Fellowship in Pharmacology. She served as an intern in the Mt. Sinai Hospital, 1948-49 and after July 1, 1949 will be at the Montefiore Hospital, New York.

American Goiter Association

RECIPIENT OF VAN METER PRIZE AWARD FOR 1949

The Van Meter Prize for 1949 was awarded to *Dr. William McKendree Jefferies* of the Massachusetts General Hospital, Boston, Massachusetts, for his essay on "Studies of the Relationship of the Thyrotropic, Exophthalmic and Fat-Mobilizing Principles of Pituitary Extract."

Dr. Jefferies was born in 1915 in Richmond, Virginia. He received the B. A. degree summa cum laude from Hampdon-Sydney College in 1935 and the M. D. degree from the University of Virginia in 1940. He served as an intern at the Massachusetts General Hospital, 1940-42. He then entered the U. S. Army Medical Corps, went overseas as a Flight Surgeon in the India-China Division of the Air Transport Command in 1943 and was discharged in February 1946 as a Lt. Colonel. From 1946 to the present he has been a clinical and research fellow in medicine at the Massachusetts General Hospital, first in the Thyroid Clinic and then in the Endocrine Clinic. During this period he was the recipient of a special fellowship fund of the American College of Physicians and of an American Cancer Society fellowship granted by the Committee on Growth of the National Research Council. After July 1, 1949 he will be at Western Reserve University Medical School, Cleveland, Ohio.

FIRST HONORABLE MENTION

First Honorable Mention was given to *Dr. Malcolm M. Stanley* of the Joseph H. Pratt Diagnostic Hospital, Boston, Massachusetts, for his essay on "The Direct Estimation of the Rate of Thyroid Hormone Formation in Man. The Effect of the Iodide Ion on Thyroid Iodine Utilization."

Dr. Stanley was born in 1916 in Henderson, Kentucky. He received the A. B. degree from Centre College of Kentucky in 1937 and the M. D. degree from the University of Louisville (Ky.) School of Medicine in 1941. After serving as interne and assistant resident at the Gallinger Municipal Hospital, Washington, D. C., 1941-43; as assistant and then chief resident in medicine at the Evans Memorial Hospital, Boston, and as assistant and then instructor in medicine at Boston University School of Medicine, 1943-46, he went to Joseph H. Pratt Diagnostic Hospital, Boston. During 1946-48 he was the recipient of a fellowship of the American Cancer Society sponsored by the Committee on Growth of the National Research Council, was a research associate in endocrinology, 1948-49, and became a staff member (gastro-enterology) in 1949. At Tufts College Medical

during their stay in this country for conferences with them and their preceptors, thus to follow their progress. They will also be visited at intervals after their return to their home institutions in an effort to evaluate the end results of their training and to offer any possible assistance to improve teaching, research and practice in the field of internal medicine in their respective countries.

FELLOWSHIPS FOR LATIN-AMERICAN PHYSICIANS

The American College of Physicians and the W. K. Kellogg Foundation, with the cooperation of the U. S. Department of State and of medical schools in the U. S. A., Canada and Latin-American countries, will shortly inaugurate a program of postgraduate medical fellowships. Outstanding young physicians will be nominated to the College and Foundation by local committees in the countries to the south, and those to whom fellowships are awarded will be brought to this country for a year or more of special training. It is anticipated that the first fellows will begin their studies during the autumn of 1949.

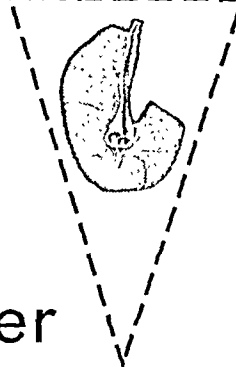
Eligibility requirements include citizenship in the country from which application is made and familiarity with its culture and economy, graduation from an acceptable medical school and completion thereafter of an internship of twelve months or more, ability to use the English language, and assurance of a subsequent teaching affiliation with a medical school in the native country. Those needing some training in English will be assigned to a special course for the purpose in the United States.

Designed to stimulate progress in the teaching of internal medicine and research, and to help the most promising young doctors of medicine in these countries to prepare for teaching and research careers in their native countries, the program also will serve to increase understanding among the American republics by serving as a medium for the exchange of knowledge and acquaintanceships.

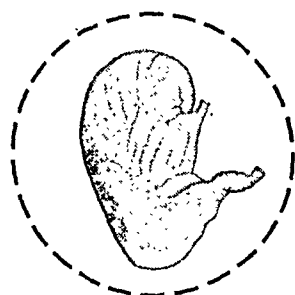
The American College of Physicians, operating through its Committee on Fellowships and Awards, will undertake to arrange a suitable program of study in internal medicine or its subspecialties, such as cardiology, gastro-enterology, etc., in widely recognized medical education centers in this country, and to place the fellows appropriately under preceptors in these institutions.

The W. K. Kellogg Foundation will provide each fellow with a monthly stipend adequate for his basic living costs, an allowance for necessary travel within this country or Canada, and will defray the tuition for courses recommended by the preceptor and approved by the sponsors. In view of the pressing need of Latin-American medical libraries, the Foundation will reimburse the fellow for the cost of required textbooks on condition that they become the property of the medical school in which the fellow will teach upon his return home.

Representatives of the Foundation will visit the fellows periodically



liver



plus

stomach

equals

red blood cells



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The Journal of CLINICAL ENDOCRINOLOGY

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heat, growth, muscular work and the multiple special functions in the highly organized systems. Potassium is the chief basic ion (or cation) of the cells, its concentration being variously estimated between 140 and 160 mEq. per liter of cell water (1). Outside the cells, in the vascular and perivascular spaces, sodium is the chief cation, and normally potassium exists here in a concentration of only 4.0 to 5.5 mEq. per liter. Between the two compartments, there must be maintained ionic, osmolar and acid-base equilibria. Under certain abnormal circumstances, as will appear later, some sodium can invade the cells and replace potassium there; but available evidence indicates that there is a reduction of the functional efficiency of the cells when this has occurred.

It has recently been demonstrated by Folk, Zierler, and Lilienthal (2) that the potassium concentration of the extracellular fluids is accurately reflected by the plasma concentration of this element, according to the Gibbs-Donnan equilibrium. This is an exceedingly fundamental piece of information, for unless the readily available plasma could be used for potassium determination, one would have no way of knowing the concentration of potassium in the fluid actually bathing the cells.

The concentration of potassium in the extracellular compartment is clearly the resultant of how much of this element comes in and how much goes out. Potassium can enter this compartment by way of the gut—absorption of ingested potassium—or it can emerge from the cells. Potassium can leave the extracellular space by entering the cells or by excretion in the urine. Only in diarrheal states are significant amounts of potassium lost by way of the stools. Gastric juice (and perhaps juices of the small intestine as well) contains potassium in concentration several-fold greater than the plasma. We have found this true in patients with and without free hydrochloric acid, and it has only been in patients suffering from very low serum potassium levels that gastric juice has contained less than 10 mEq. per liter; usually from 15 to 25 mEq. (3). Thus excessive vomiting or prolonged gastric suction might prove to be ways of losing considerable quantities of potassium. Homeostatic mechanisms are so delicately arranged that it requires a major functional disturbance to alter the concentration of potassium in the extracellular fluids from its normal range. When elevation or depression of the potassium concentration does occur, however, serious consequences ensue.

It has long been known that alterations in the potassium concentration in the fluids bathing the heart cause disturbances in cardiac mechanisms. Ringer (4), in developing his solution with which to perfuse the isolated mammalian heart, found that either too high or too low a concentration of potassium interfered with the normal function. Development of electrocardiography, together with accurate methods for the measurement of

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THE USE OF POTASSIUM IN THERAPY*

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Baltimore, Maryland*

THE title of this discussion should perhaps be broadened to "the background for the use of potassium as a therapeutic agent, and some clinical experiences with potassium administration." In the case of a pharmacologic agent, foreign to the organism, one might begin such a topic with the functional alterations resulting from administration of the substance. But in the case of potassium, one is dealing with an element which is present in large quantity in the animal organism, and which constitutes the major basic element of chemical structure of the cell. Thus a review of current knowledge of the metabolism of this substance, both normal and abnormal, is essential for an intelligent approach to its use as a therapeutic agent. It warrants emphasis that, though the present discussion will highlight the activities of this single structural element, other components such as protein, phosphorus, sulfur and magnesium, deserve equal consideration in the preservation of the functional efficiency of the cell.

The cell is the basic structural unit of the human organism. Within its confines enzymatic and other metabolic processes furnish the energy for

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Read in abstract before the American Clinical and Climatological Association, Hot Springs, Virginia, October 12, 1948.

** Research Fellow in Medicine.

ST segment produces the change. The other changes in the ST segments seen with low potassium are also of differential value. Digitalis and myocardial disease influence the Q-T interval and these factors should be considered in interpreting this measurement.

The second change is lowering or inversion of the T waves and in the third stage the ST segments sag. In the final stage the take-off of the ST segments is depressed and there is a slow staircase-like rise to a low late T wave. This final stage is seen with potassium levels in the range of 1.5 milliequivalents per liter or lower.

With high serum potassium the first change seen in the electrocardiogram is elevation of the T waves, and this is followed by a decrease in the amplitude of the R waves. The third stage is characterized by auricular arrest and no P waves are seen. At slightly higher levels the ST segments become depressed and the QRS duration increases. In the terminal stage, with potassium levels in the range of 9.5 to 10 milliequivalents per liter, the QRS becomes so prolonged that it blends with the elevated T wave to give a biphasic curve. These broad "sine wave" complexes may occur with complete irregularity as the auricular pacemaker has dropped out.

Peripheral, as well as cardiac, neuromuscular mechanisms are disturbed as the result of abnormal concentrations of potassium. Curiously enough, the neurologic manifestations are much the same whether the potassium be high or low. Sensory disturbances are minimal, usually with complaints of only mild paresthesias of the extremities. Motor phenomena are striking; first weakness and then flaccid paralysis of the extremities, sometimes of the ascending or Landry's type. The trunk and cranial nerves are rarely involved but weakness of the respiratory muscles results in a shallow, regular, rapid pattern which is distinctive. However, these peripheral neuromuscular defects are late manifestations of hyper- or hypokalemia and are always preceded by electrocardiographic changes. Conversely, in recovery from the dysfunction of hypokalemia, the electrocardiogram shows reversion to normal soon after return of the serum potassium to normal, whereas the paralytic phenomena pass far more slowly and, indeed, may persist for several hours.

Experimental work on potassium metabolism in animals has yielded much important information, of which space permits summarization of but a few points. Orent-Keiles and McCollum found that, on diets very low in potassium, rats died within three weeks (17). Postmortem analysis revealed the potassium content of heart, skeletal muscle and kidney to be greatly reduced, as much as one-third; amazingly enough, the liver potassium was normal. In these animals Follis found necrosis and scarring of the heart

potassium on small samples of biologic materials, brought about recognition of the changes in cardiac mechanisms coincident with high and low concentrations of potassium in the serum (5-16). The almost universal availability of the electrocardiogram renders the cardiographic record the most practical method to the clinician for the detection of hyper- and hypokalemia. For the benefit of those not familiar with the diagnostic points involved, a brief schematic series of sketches is shown (Fig. 1).¹

PROGRESSIVE EFFECT OF
LOW SERUM K ON EKG

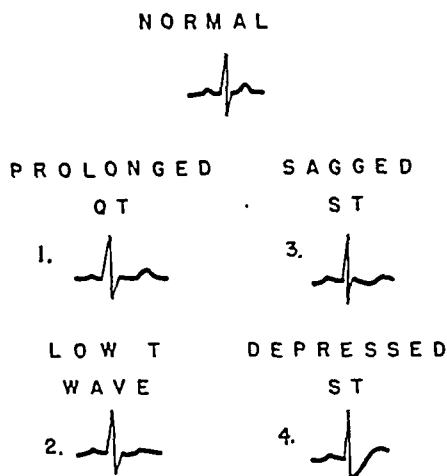


FIGURE 1

Effects of potassium on the human electrocardiogram: The changes in the electrocardiogram produced by abnormalities of the serum potassium are characteristic and easily recognized. As the serum potassium is lowered, prolongation of the Q-T interval is the first change seen in the electrocardiogram. The Q-T interval varies with the heart rate, but at average heart rates it should be less than one-half the R-R interval and this can be used as a rough upper limit of normal. It is well known that hypocalcemia prolongs the Q-T interval, but Nadler (14) and others have shown that the low potassium effect is independent of serum calcium. Nadler suggests that the Q-T interval prolonged by low potassium can be distinguished from that prolonged by low calcium. The low broad T wave of hypokalemia is primarily responsible for the prolongation of the Q-T interval, while in hypocalcemia the T wave is normal and the lengthened

¹ For these sketches and the description in the succeeding paragraphs, we are indebted to Dr. R. S. Ross.

CLINICAL OBSERVATIONS

It has been known for more than ten years that some abnormality in potassium metabolism plays an important role in the attacks of familial periodic paralysis (26). The serum potassium is found to be lowered during the paralytic phases, and attacks may be induced by such physiologic "potassium-lowering" procedures as a glucose test meal, administration of insulin or by epinephrine (27, 28, 29). Furthermore, both spontaneous and induced episodes are promptly cured by potassium salts. The exact nature of the underlying physiologic defect in this condition is still unknown. There is no apparent deficiency of cellular potassium.

Hypokalemia, sufficiently severe to produce paralytic phenomena, has been observed to follow overzealous administration of desoxycorticosterone to patients with Addison's disease (30). Recovery followed the oral use of potassium chloride. Here, of course, there was presumptive depletion of cellular potassium, as is found in the experimental animal poisoned with desoxycorticosterone (19, 24). In the normal adult economy, almost the same quantity of potassium is excreted in the urine as has been ingested. In most types of renal insufficiency, so long as urinary volume is maintained, the capacity to excrete potassium remains adequate to cover the requirements; and clinically significant deviations from the normal concentrations of potassium in the serum do not occur unless unusually heavy loads are thrown on the mechanism, such as administration of potassium salts. In oliguric uremia, elevation of serum potassium is not unusual; indeed death from hyperkalemia is probably more common than is generally recognized. Occasionally a type of disturbed renal mechanism is met which results in the urinary output of greater quantities of potassium than normal; there is thus brought about a depletion of potassium with the usual neurologic and electrocardiographic signs thereof (31, 32). Prompt recovery from the immediate presenting symptoms occurs with administration of potassium salts.

In the remaining examples of hypokalemia to be discussed, there are associated profound disorders of nutrition. As a background for an understanding of these cases, it would therefore seem wise to review some of the data on the metabolism of potassium in starvation and recovery therefrom. When no food is eaten, there is expenditure of cellular elements by the body for energy, heat and repair. In Benedict's experiment (33), in which his professional "faster" took nothing but water for thirty days, the amounts of nitrogen, potassium, phosphorus and sulfur that appeared in the urine closely paralleled the relative proportions of these elements in muscle protoplasm. During his month of starvation, Benedict's patient "consumed" and excreted between 15 and 20 per cent of his protoplasmic

muscle fibers, as well as necrosis of renal epithelium with tubular dilatation (18). Low serum potassium, low muscle potassium and myocardial necroses have been produced also by poisoning with desoxycorticosterone, presumably in large part as the result of the potassium diuresis effected by this steroid (19, 20, 21).

Darrow and his co-workers (22, 23, 24), using a variety of techniques to induce lowering of the cellular potassium, confirmed previous evidence that there exists a reciprocal relationship between sodium and potassium in muscles; that is, whenever muscle potassium was lowered, there was a corresponding increase in the sodium content. They found in their rats also that whenever a decrease in muscle potassium of more than 10 per cent was present, there was invariably a significant lowering of the concentration of potassium in the serum. Of considerable interest was their observation that potassium administered to potassium depleted animals was retained; whereas, when potassium was injected into normal rats, muscle content was increased only momentarily, and the balance of potassium was soon actually negative. Recently Darrow has pointed out that in their experimental animals there existed a high degree of correlation between the concentration of bicarbonate and chloride in the serum, and the muscle content of sodium and potassium. High serum bicarbonate and low serum chloride invariably accompanied low muscle potassium and high muscle sodium, whether the latter state had been induced by low potassium diet, desoxycorticosterone or the administration of sodium salts (25). It was emphasized by Darrow and co-workers that these predictable relationships were found to apply only after "biological equilibrium" had been reached, a term implying that renal function was being normally carried out. Thus, as in practically every pathologic state, the presence of renal insufficiency greatly complicates interpretation of the chemical anatomy of the body fluids.

To summarize the data presented thus far: There is experimental evidence that 1) the organism is incapable of adequately withholding potassium in the face of a prolonged dietary deficiency of this element; 2) that this potassium deficit is accentuated by coincident administration of sodium salts or by desoxycorticosterone; 3) that reduction of cellular potassium below a certain point is dangerous not only in causing muscular (myocardial) necroses, but that serum potassium is also lowered. Recognition of hypokalemia may be obtained through electrocardiography, and is to be suspected in symmetrical peripheral motor palsies with little or no sensory changes. Potassium depletion should be suspected when, in the presence of normal kidney function, there is an unexplained high serum bicarbonate and low chloride concentration.

The exhaustive studies on potassium and sodium interrelationships made by Darrow's group (20, 22, 23, 24, 25), and the knowledge that relatively large quantities of potassium were lost in diarrheal stools, led them to the use of potassium salts parenterally in the treatment of infantile diarrhea. Previously, standard therapy had included only sodium salts. In the first sizeable group of cases treated by the newer method, the mortality was reduced from 32 per cent to 6 per cent (41).

Diabetic acidosis

Since, in the studies on convalescence (37), our attention was being directed toward the provision to the cells of elements that had been depleted and might be accepted and beneficial, in 1945 potassium salts were added to the infusions given to patients with diabetic acidosis. There was, at the time, no conscious effort to forestall the development of hypokalemia. It had been known, since the classical studies of Atchley, Loeb, Benedict, Richards and Driscoll (42) on diabetic volunteers, that during the development of insulin-withdrawal acidosis, diuresis of large quantities of potassium, nitrogen, phosphorus and other elements occurs, much like the pattern of starvation. The study by Atchley's group had also demonstrated that, with resumption of insulin, there was a sharp retention of all the previously lost elements, especially conspicuous in the case of potassium. Their patients had, of course, been fed constant diets during the experimental periods, and insulin was resumed when the metabolic situation seemed precarious. The first few patients with diabetic acidosis studied here, to whose intravenous therapy potassium salts were added, manifested strongly positive potassium balance without elevation of serum potassium, an indication that the majority of the administered potassium ions had been taken up by the cells (43). When it became apparent from the papers of Holler (44), Martin and Wertman (15), Frenkel, Groen and Willebrands (45), Nicholson and Branning (46), and Logsdon and McGavack (47) that, with the current therapeutic regimens, patients recovering from diabetic acidosis might be seriously endangered by the development of hypokalemia, the quantities of potassium given to our patients were increased to see just how much the cells might be capable or "desirous" of accepting. We were quite unprepared for the enormous positive balances shown by some of our cases (43). In one instance, as much as 35 grams of potassium chloride (467 mEq. of potassium) was injected into a patient in eighteen hours; and less than 100 mEq. potassium appeared in the urine. During this time the serum potassium was constantly subnormal. It has been our impression that patients given large doses of potassium and far less sodium than with currently common therapeutic regimens, have profited greatly, not only in the prevention of the dangerously low levels

mass. Gamble, Ross and Tisdall's studies on children also showed great losses of intracellular elements during fasting (34). Observations made during World War II indicated that the same type of parallel expenditure of cellular elements accompanied brief starvation periods as well as periods of graded undernutrition, provided the diet was kept *qualitatively* constant (35). But during recovery—that is, when these patients were fed full diets—it was found that potassium was retained sooner and in greater quantity than was nitrogen, even though nitrogenous foods were offered liberally. A similar retention pattern was noted when obese patients were starved for four days and then fed 1200 calorie diets (36).

During experiments with total intravenous feeding, it seemed logical to several members of the group studying the metabolic aspects of convalescence (37) to include salts of potassium along with glucose and the nitrogenous pabulum of protein hydrolysates, thus at least offering to the cells another of their normal constituents. It was found that potassium was accepted under these circumstances, and often in considerable quantities. Our group, since 1944, have felt it rational to add from 3 to 8 grams of potassium chloride per day (40 to 110 mEq. of potassium), with corresponding reduction of sodium chloride, to all total intravenous feeding mixtures (except in patients with anuria or renal insufficiency of the potassium-retaining type).

Stewart and Rourke (38) had noted in 1942 that, in postoperative patients fed only parenterally, more potassium was lost in the urine of a patient given normal saline than was lost by a patient to whom only glucose solutions were given. Mason and Howard (39) had noted that sodium and chloride were retained postoperatively in amounts seemingly far greater than were needed for simple repletion of the extracellular space; and, since exaggerated retention of sodium chloride occurred during simple oral feeding after brief starvation periods (36), it seemed likely that avidity for sodium and chloride was a phenomenon of undernutrition. If then, in undernutrition, there is depletion of, along with other things, potassium; and administration of sodium causes great retention of sodium, and potassium diuresis (40), why were we not frequently observing hypokalemia among undernourished patients who were being given only sodium chloride and glucose parenterally? The answer is that we were seeing such cases but not recognizing them; for the significance of much of these data was not realized at the time.

Infantile diarrhea

The administration of potassium salts with the avowed purpose of replenishing cellular supply or preventing further vital depletion was reported from Darrow's laboratory in the treatment of infantile diarrhea.

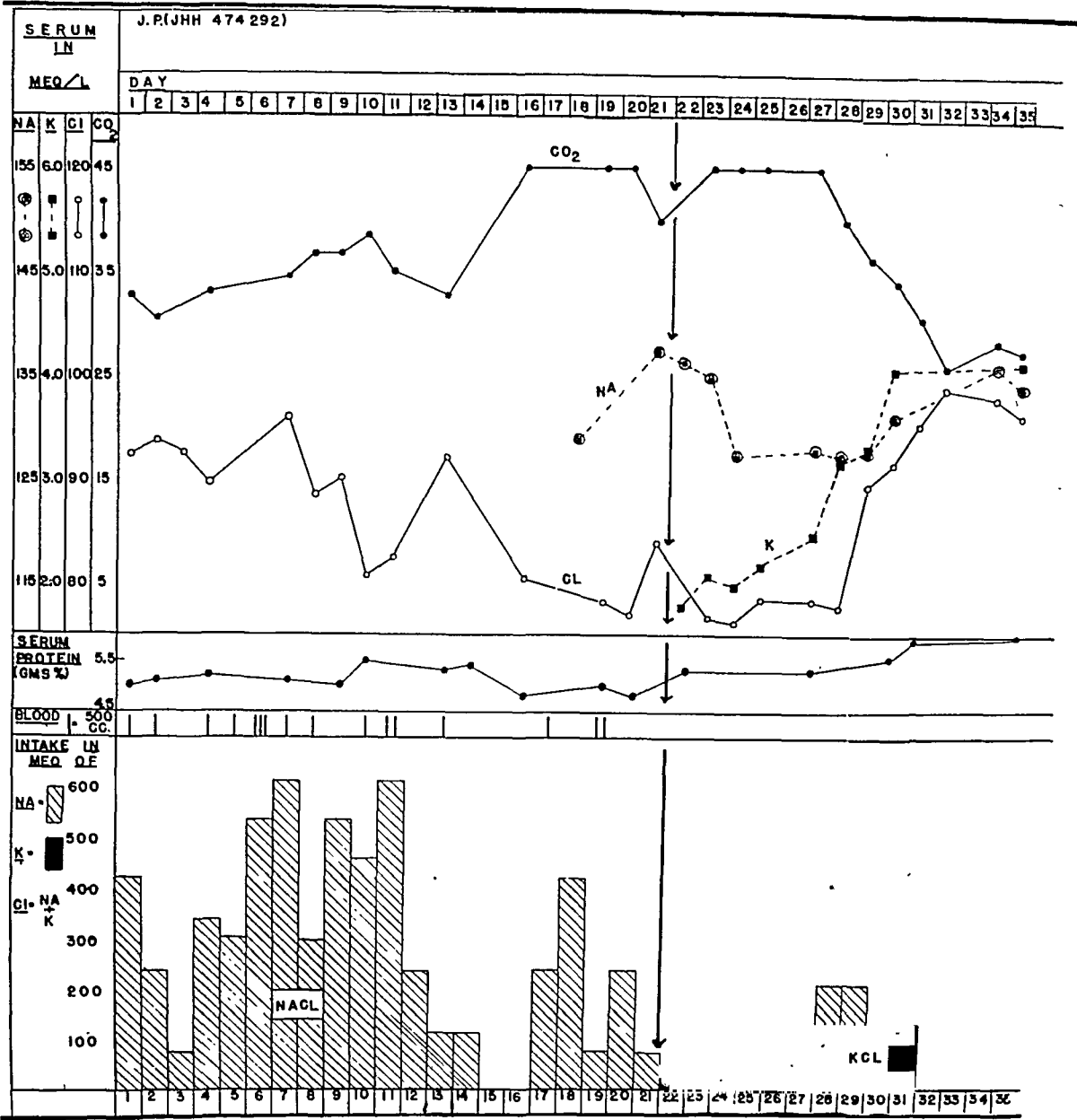


FIG. 2. Electrolyte pattern of serum in case J.P. Intake of sodium prior to administration of potassium was over 6000 mEq. On day 28 and 29, 145 mEq. of sodium was given. Total intake of potassium was 991 mEq.

ing was stopped, and the patient was given 10 grams of potassium chloride orally, together with a routine surgical liquid diet.

It is most unfortunate that urinary incontinence prevented the collection of balance data on this patient. The serum CO₂ determinations prior to October 8 were recorded by the laboratory simply as "greater than 40 mEq." and the actual height reached is unknown. The long latent period (six days) before either clinical or laboratory improvement became evident was a source of discouragement, but subsequent cases have led us to expect this phenomenon. There seemed to be adequate urinary volume throughout this period; no edema was ever apparent, nor was there evidence either of shock or cardiac

of potassium which sometimes develop, but the patients have seemed to show a quicker recovery to fitness and regulation. There has also been in the patients so treated a conspicuous absence of edema and hypoproteinemia, which were common aftermaths of our former therapeutic procedures.

Chronically ill, undernourished patients

As previously mentioned, for several years patients under the nutritional study by our group have been given parenteral feeding mixtures which included moieties of potassium. It seemed likely that, among debilitated and depleted persons to whom only saline, glucose and perhaps plasma or amino acids were given, there might be found a fair number who would show evidences of hypokalemia. Last summer, with the aid of surgical colleagues, patients of this type were sought. The following cases are representative of this group:

J.P., J.H.H. #474293, was a suspect because of an unexplained high serum bicarbonate and a low serum chloride, against which efforts at correction by normal saline and ammonium chloride had been unavailing. He was a man aged 53, who had been ill for over a month before entering the Johns Hopkins Hospital. There had been constant abdominal pain which permitted only minimal ingestion of food. Weight loss had been greater than 20 pounds. After hydration with sodium chloride and glucose solutions, abdominal operation was performed on September 20, and there was found pancreatic necrosis, abscess formation, perforated gall bladder and infarction of the omentum. During the postoperative course, practically nothing could be taken by mouth and parenteral feedings consisted of glucose, saline, plasma and whole blood (Fig. 2 and Table 1). As the serum chloride was noted to be falling and the bicarbonate rising, sodium chloride therapy was pushed, and ammonium chloride added—without improvement (October 1–4). On October 5, examination in consultation showed the patient to be extremely asthenic; respirations were shallow but only slightly more rapid than normal; peripheral reflexes were barely obtainable; blood pressure was normal, and there were no evidences of “shock” or heart failure. The cardiogram disclosed definite evidence of hypokalemia.

On October 6, the morning that potassium therapy was begun, the serum potassium was found to be 1.8 mEq. per liter. A mixture of glucose and “Elamine,”² to which potassium chloride was added, was given by vein each day over periods of eight to ten hours (Fig. 2 and Table 1). The first day 4 grams of potassium chloride was given. No sodium was included in the mixture except on October 12 and 13, when 500 cc. of normal saline was added.

There was no apparent change in the patient's condition, nor in the chemical pattern of the plasma until October 12, the seventh day of potassium therapy, at which time there began a dramatic improvement in both. He began to feel progressively stronger, appeared more alert and reflexes became readily obtainable. On October 14 the serum potassium had reached a level of 4 mEq. per liter, chloride was rising and CO₂ falling; serum sodium, which had fallen during the intravenous potassium period, was rising. Intravenous feed-

² Elamine is a protein hydrolysate which contains minimal quantities of sodium chloride. The material was furnished through the courtesy of the Interchemical Corporation.

TABLE 2

E.A. (J.H.H.#467811)

Date	Intake							Output							Balance						
	Na	K	Ca	Cl	P	N	Fluid cc.	Na	K	Ca	Cl	P	N	Fluid cc.	Na	K	Ca	Cl	P	N	Fluid cc.
Nov. 3-4	101	112	423	149	915	12.5	3250	99	44	94.5	147	356	9.9	1205	+ 2	+68	+329	+ 2	+559	+2.6	+199.3
4-5	101	112	423	149	915	12.5	3250	74	36	182.0	111	312	9.2	1000	+27	+76	+241	+ 38	+603	+3.3	+2250
5-6	101	112	423	149	915	12.5	3250	89	21	154.0	92	75	6.4	860	+12	+91	+269	+ 57	+840	+6.1	+2390
6-7	101	112	423	149	915	12.5	3250	33	38	109.0	49	178	8.4	950	+68	+74	+314	+100	+737	+4.1	+2300
7-8	93	103	389	138	843	11.5	3000	75	44	141.0	62	342	8.5	1050	+18	+59	+248	+ 76	+501	+3.0	+1950
8-9	96	106	403	143	852	11.9	3100	102	74	280.0	100	328	10.4	1640	- 6	+32	+123	+ 43	+524	+1.5	+1460
9-10	101	112	423	143	915	12.5	3250	104	79	232.0	108	462	9.3	1540	- 3	+33	+191	+ 35	+453	+3.2	+1710
10-11	101	112	423	143	915	12.5	3250	119	89	238.0	140	644	10.6	2060	-18	+22	+185	+ 3	+271	+1.9	+1170
11-12	101	125	423	163	915	12.5	3250	79	65	180.0	95	526	7.6	1200	+22	+60	+243	+ 68	+389	+4.9	+2030
12-13	101	138	423	176	915	12.5	3250	105	88	240.0	129	552	9.7	1380	- 4	+50	+183	+ 47	+363	+2.8	+1870
13-14	101	138	423	176	915	12.5	3250	86	114	288.0	140	567	11.4	1620	+15	+24	+135	+ 36	+348	+1.1	+1630
14-15	101	138	423	176	915	12.5	3250	77	102	288.0	134	603	11.6	1340	+24	+36	+165	+ 42	+312	+0.9	+1910
15-16	97	133	406	169	878	12.0	3150	20	102	216.0	118	434	10.1	1160	+77	+31	+190	+ 51	+444	+1.9	+1990

TABLE 1

J.P. (J.H.H. #474293)

Date	Serum Values							Intravenous Intake			Remarks	
	Cl mEq./L.	CO ₂ mEq./L.	Na mEq./L.	K mEq./L.	P mg./100 cc.	Ca mg./100 cc.	Total protein Gm./100 cc.	NPN mg./100 cc.	Blood cc.	Na mEq./L.		K mEq./L.
1948												
9-15	92.5	32.8					5.0	25	500	422.37		Admitted; sick for six weeks, little or no food, nausea or vomiting
9-16	93.8	30.8					5.1	23	500	240.85		Levin tube Operation
9-17	92.6						5.2 (2.0)	22	500	76.95		
9-18	80.9	33.0							500	316.3		
9-19									500	307.8		
9-20	96.0	34.7					5.1	23	1500	538.6		
9-21	88.6	30.8							250	615.6		
9-22	90.3	36.8					5.0	14	750	300.1		Bilateral ligation of superficial femoral veins
9-23	80.7	38.5					5.5	22	150	461.7		
9-24	82.0	35.1						22	1350	615.6		
9-25							5.3	30	500	230.8		
9-26	92.2	32.8				7.8	5.3	20		115.4		
9-27												
9-28												5985 mEq. Na+Cl to date Potassium therapy begun Miller-Abbott tube started
9-29	80.8	40.0					4.8	28	500	240.85		
9-30			120.0							423.30		
10-1									1000	79.90		
10-2	78.4	40.0					5.0 (2.8)	22				
10-3												
10-4	77.0	40.0	137.5		1.6	8.7	4.8 (2.9)	22		240.85		773 mEq. K to date Oral administration begun; i.v. stopped
10-5	84.0	40.0	130.3	1.8	1.0		5.3 (3.6)	21		70.90	53.6	
10-6								30			80.4	
10-7	76.8	40.0	135.0	2.1							80.4	
10-8	70.5	45.0	127.5	2.0							80.4	
10-9	78.5	45.0		2.2							80.4	
10-10												991 mEq. K to date
10-11	76.3	45.0	128.0	2.5			5.3				80.1	
10-12	77.7	40.0	127.5	3.2						76.05	131.0	
10-13	80.6	30.4	127.7	3.35	0.5					76.05	134.0	
10-14	91.8	34.3	131.2	4.1			5.6	22				
10-15	95.4	20.8			0.7	8.8	5.9 (3.0)					
10-16	90.0	26.2									131.0	
10-17												991 mEq. K to date
10-18	98.2	28.4	136.3	4.05								
10-19	90.2	27.0	133.8	4.15			6.1 (3.3)					

a colectomy was performed and the ileum brought down to the anus. A few days after operation, spiking fever and diarrhea set in. The small oral feedings that could be tolerated were supplemented by blood, plasma, glucose, saline and, on two occasions, small amounts of "Amigen"³ parenterally (Tables 2 and 3 and Fig. 3).

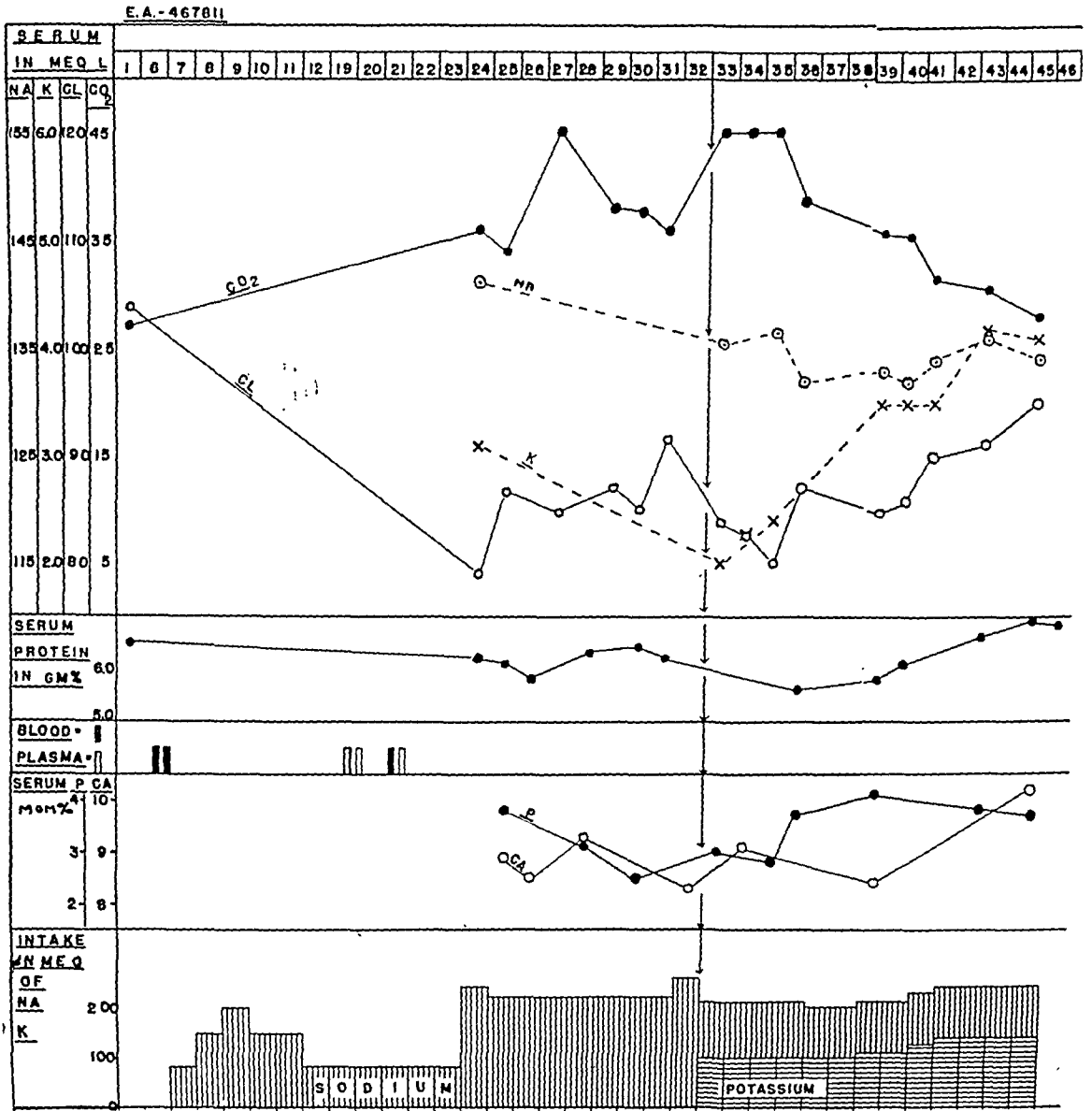


FIG. 3. Electrolyte pattern of serum in case E.A. during 46-day period. The symbols for blood and plasma equal 500 cc.

On November 2 examination in consultation disclosed elevated temperature and pulse, no respiratory distress and normal blood pressure. There was no edema or other evidence of heart failure. The patient was apathetic and weak; peripheral reflexes were practically absent; urine was normal; the cardiogram showed evidences of low serum potassium. The following morning, prior to institution of potassium therapy, the serum concentrations were as follows: potassium 2.0 mEq. per liter, chlorides 83.9 mEq. per

³ The Amigen used in these studies was furnished by Mead, Johnson & Co., through the courtesy of Dr. Warren Cox, Jr.

decompensation. Once the chemical pattern began to change, the rapidity of its return to normal was astonishing and most gratifying. Even the serum protein concentration, which had been between 5.0 and 5.3 grams per 100 cc. since September 15 despite transfusion of 7 liters of blood, rose to 6.1 by October 19, coincident with the rises in serum potassium and chloride and the fall in CO_2 . Serum phosphorus, very low when potassium

TABLE 3

E.A. (J.H.H. #467811)

Serum Values										
Date 1948	Cl mEq./L	CO ₂ mEq./L	Na mEq./L	K mEq./L	Ca mg./100 cc.	P mg./100 cc.	Tot. protein Gm./100 cc.	NPN mg./100 cc.	Hematocrit vol. %	
10-2	103.8	26.0	141.3	3.1	8.9	3.8	6.5(RI)		28	
10-7							8.6(5.4)		54	
10-25	79.0	36.0					6.2(2.1)		25	
10-26	86.8	34.3					6.1(3.3)		22	
10-27			.		8.5		5.8			
10-28	84.8	40 +								
10-29					9.3	3.1	6.3		25	
10-30	87.0	38.0								
10-31	85.0	37.6				2.5	6.4		18	
11-1	91.7	36.0					6.2(3.9)		33	
11-2					8.3				26	
Potassium Therapy Started										
11-3	83.9	40 +	135.4	2.0	9.1	3.0				
11-4	82.7	40 +		2.3						
11-5	80.2	40 +	136.6	2.4		2.8				
11-6	87.3	38.5	132.0		8.4	3.7	5.6	19.8	19	
11-9	84.9	36.8	133.0	3.5		4.1	5.8		19	
11-10	85.8	36.0	132.0	3.5			6.1	23.0	28	
11-11	89.9	31.8	134.0	3.5	10.2			20.8		
11-13	91.4	30.8	136.4	4.2		3.8	6.6		20	
11-15	94.5	28.4	134.6	4.1		3.7	6.9		21	
11-19							6.8		21	

therapy was begun, fell even lower to values of 0.5 on October 13. No phosphorus was administered to this patient; "Elamine" contains none.

E.A., J.H.H. #467811, was a 44-year-old white woman who entered the hospital on October 2, 1948. Sixteen years previously an ileostomy had been performed for ulcerative colitis, with rapid improvement in the patient's weight and strength. Aside from the annoyance of the ileostomy, the patient had no complaints. She had been on a general bland diet. Physical examination revealed no important abnormalities. On October 8

liter, CO_2 greater than 40 mEq. per liter and sodium 135.4 mEq. per liter; calcium 8.3 and phosphorus 3.0 mg. per 100 cc.

Beginning November 2, oral feeding was stopped and the patient was given by vein each day a mixture of 1 liter of 10 per cent "Amigen," 220 grams of glucose (total fluid volume 3250 cc.) to which was added 8 grams of potassium chloride. The only sodium given was that contained in the liter of 10 per cent "Amigen," approximately 100 mEq. Except for the fever, clinical improvement was prompt. The diarrhea ceased abruptly with instigation of the total intravenous feeding, and drainage from the rectum was negligible until oral feeding was resumed. There was, however, the same delay, as seen in the previous case, in return of the electrolyte pattern toward normal. It was not until November 11 (eight days later) that there was manifest an appreciable rise in serum chloride and a fall in CO_2 ; and even by November 15 the concentrations of these electrolytes and potassium could not be considered entirely normal. Again there was a coincident appreciable rise in serum protein concentration, without further administration of either blood or plasma.

On November 2, just prior to the total intravenous feeding program, the abdomen was reopened and a perirectal abscess drained. The drainage was considered to be inadequate, and remittent fever continued.

Balance data in Table 2 and Figure 4 show that a large portion of the administered potassium was retained. In the first five days there was a positive balance of 368 mEq. of potassium. In these 2 patients just cited, the analogy to Darrow's observations in his potassium depleted rats is striking. Both patients had suffered long periods of undernutrition in which potassium must have been lost in considerable quantities along with other intracellular elements. Both had received relatively large amounts of sodium chloride. Potassium had probably been further depleted by the diarrhea in the second case. Serum potassium was low, the chlorides low and the CO_2 elevated. Administered potassium was retained in the second case and presumably in the first case also, just as Darrow had found when potassium was given to the depleted rats (25). It was our impression that the delayed rise in serum potassium and reversal of the chlorides and CO_2 concentrations were expressions of the fact that the cells were avidly accepting the proffered potassium; and until the cellular compartment had been repleted up to a certain point, there was no reflection of the improvement in the chemical anatomy of the extracellular compartment. It is striking that during the period of therapy with potassium chloride, the second patient (E.A.) retained chloride throughout the entire period,⁴ more in the earlier days when the serum chloride concentration was remaining relatively stationary than later (November 9 to November 13) when serum chloride began to rise. Sodium balance, except on November 5—on which day there is some reason to believe some urine may have been lost—was essentially in equilibrium. It seems likely that the administration of

⁴ No attempt was made to allow for sweat in any of these experiments; in none of the patients was undue sweating a feature.

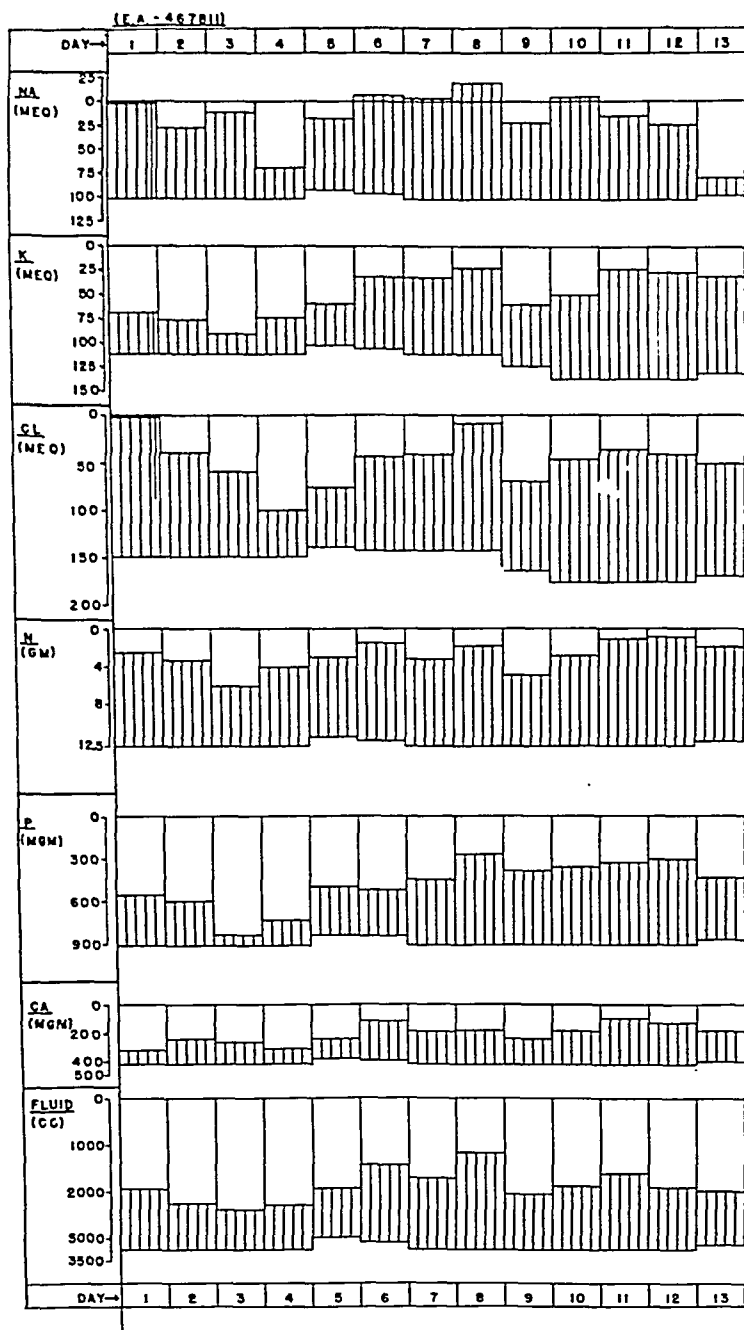


FIG. 4. Balance data of patient E.A. during administration of potassium. Day 1 of this figure is day 33 of Figure 2. Intake is represented by the distance from the lowest line to the zero line. Output is represented by the hatched area. Positive balance is indicated by the clear area below the zero line; negative balance by the hatched lines extending above the zero line.

the major portion having been lost on days 1 and 2. These dogs had pylorectomy performed by Dr. Scott on the day of institution of treatment; but since nothing was given by mouth and there was no vomiting, the operation probably contributed no added burden to the animals after the first two days. It was next decided to see what effect exactly the same procedure would have on dogs who had previously been starved for ten days but allowed free access to drinking water. During starvation, urine was collected so that pretreatment losses were known. One such dog was given sodium chloride and glucose only as above; two other dogs were given a mixture consisting of glucose 100 Gm., sodium chloride 2 Gm. and potassium chloride 6 Gm. in 2,000 cc. of water.

After two weeks the dogs given the potassium chloride seemed healthy and frisky; the blood morphology and chemical pattern of the serum were essentially normal. However, on the eighth day the dog given only sodium chloride and glucose was drowsy, apathetic, too weak to stand, slightly edematous and the chemical pattern of the serum was as follows: potassium 2.9, sodium 144, chlorides 114, CO_2 23.2 mEq. per liter, nonprotein nitrogen 27 mg. per cent and hematocrit 46 vol. per cent. The dog was found dead in his cage two hours later.

Analysis of the balance data on these 2 dogs showed the surprising fact that from the beginning of the two types of total intravenous feeding, the over-all negative balance of potassium in the healthy dog (given potassium chloride) was only 150 mEq. less than in the dog that was given sodium chloride and apparently died of hypokalemia. These studies are being continued in an effort to determine the influence of diarrhea, vomiting and other factors in addition to undernutrition on the development of the hypokalemia.

METHOD OF ADMINISTRATION OF POTASSIUM

Despite our admitted ignorance of the exact circumstances leading to potassium deficit, it seems clear that patients are not infrequently met to whom the administration of potassium as a therapeutic agent is advantageous and sometimes a life-saving procedure. When the conditions are suitable for oral administration, obviously this route is to be preferred. A solution of potassium chloride containing 40 to 80 mEq. of potassium per liter (3 to 6 grams of potassium chloride) given in 50 to 100 cc. amounts with or without a fruit juice or some other carrier has seemed entirely efficacious. We have also used one of the meat juice preparations, of which more will be said later, which contains a very high concentration of potassium and which is quite palatable to many persons. But in situations of emergency or when gastro-intestinal dysfunction precludes oral administration, a parenteral route must be used. The plasma reaching the heart must

the potassium prevented retention of sodium, which previous experience of our own and that of others would have led us to expect. In E.A. the serum phosphorus concentration remained at all times within normal limits, perhaps because more phosphorus was administered—the 10 per cent “Amigen” solution containing 915 mg. phosphorus per liter. In any event, the over-all balance of phosphorus was strongly positive for the first five days, there being a total of 3,240 mg. phosphorus retained.⁵ Comparing the nitrogen retained over the same period (19 grams), it may be seen that nearly three times more phosphorus was retained than the usual ratio of nitrogen to phosphorus in muscle protoplasm; and that the potassium retained was six times as great as the usual potassium to nitrogen ratio in muscle.

EXPERIMENTAL OBSERVATIONS

Other patients, with similar nutritional background, plasma chemical pattern and response to potassium therapy have been observed. One could, however, only guess at the degree of depletion of various elements in such patients, and it seemed wise to turn to animal experimentation in an effort to determine the various factors which might play predominant roles in the production of this type of clinical picture. Last fall, with the help of Dr. H. W. Scott and Mr. Robert Carroll of the Surgical Department, the authors and Miss Marjorie Foote began some observations on dogs. Brief mention of preliminary experiments now in progress follows:

The technique of total intravenous alimentation was used. The apparatus adopted is a modification of that developed by Dr. Rhode (48) of Philadelphia. Fluid mixtures are introduced into the jugular vein continuously. Appropriate bandaging and harness prevent the dog from pulling out the tubing. By an overhead trolley and a series of counterweights the dog may move about the cage or lie down at will without strain on the tubing. Rate of flow is controlled by the usual drop method. By this technique dogs have been kept in a state of good nutrition for as long as 80 days (48).

The first experiments, performed as base-line controls, consisted in giving healthy dogs heavy loads of sodium chloride (2 liters of 0.9 per cent sodium chloride per day) to which 100 grams of glucose had been added. Though the dogs developed obvious edema on the seventh day, the serum potassium did not fall, nonprotein nitrogen and serum sodium did not rise, chloride rose to 122 mEq., CO₂ concentration fell to 15 mEq., serum protein fell from 6.83 to 4.25 Gm. per 100 cc., and there was no conspicuous clinical deterioration of the animals. We were struck by the small amount of potassium lost, a total of 72 mEq. of potassium over the fourteen days,

⁵ On November 5, there may have been a portion of urine discarded; even so, the ratio between nitrogen and phosphorus and potassium would probably be but little distorted; hence, the figures are given as they stand.

have a piece of meat or a whole chicken boiled down for us until all the "essence" was out of the meat. This was given to us for its restorative power. As a medical student, J.E.H. scorned the stuff, believing that its sole virtue lay in its sodium chloride content. For many years meat extracts have been given by practitioners to their convalescent patients and taken themselves with satisfaction. Testimonials by these physicians have generally been scoffed at or ignored by the scientific medical world. Broth, as ordinarily prepared for the table, contains approximately 25 mEq. potassium per liter; concentrated stuff such as our home-made meat stock must contain far more. In our laboratory, analysis of a well-known meat juice preparation (Valentine's) showed it to contain 1,215 mEq. potassium to the liter. Four tablespoonfuls of this material contain almost as much potassium as does an ordinary day's diet.

A comment in passing, about the correction of abnormalities in the chemical anatomy of the plasma, when such are met. The usual impulse is to set about correcting all of them as soon as possible. One wonders if this may not sometimes be unwise. There are multiple factors at play which eventually fix the concentration of the various substances in the extracellular compartment. But ultimately the machinery of the kidneys and the cells of the rest of the tissues set the pattern, for the extracellular compartment is inert save for the red blood cells. It seems not unlikely that under circumstances of depletion through illness or inanition, the structure within the cells may be so altered that an environment which we consider "normal" might in reality be very unsuitable. The indiscriminate administration of various materials into the system, be they plasma, sodium, chloride, phosphate or what not, in an effort to restore to the *usual* plasma pattern, may, at times, actually put the cells at a greater disadvantage than that under which they already labor. Perhaps our advocacy of potassium as a therapeutic agent lays us open to a charge of this guilt. But if less concern were wasted on the extracellular environment and more on what this means in terms of the cellular situation and cellular needs, it seems likely that our therapeutic procedures would be more efficacious.

In closing it would be well to return to the initial remarks and repeat that in highlighting the metabolism of a single element, there have been ignored other and equally important intracellular constituents which are integrated in the highly complex intracellular mechanisms. Each of these probably deserves an equal amount of attention. As our knowledge grows, these remarks will doubtless seem inconsequential and immature. They are offered as a temporary glimpse of what will surely become a much broader view, when researches in intermediary metabolism can be more closely correlated with clinical conditions.

not contain more than 7 mEq. potassium per liter. This will depend upon not only the concentration of potassium in the administered fluid but also the rate at which it is injected into the vein. Our patients have received solutions containing between 40 and 90 mEq. of potassium per liter at rates not greater than 12 cc. per minute (180 drops per minute). Usually the rate has been about 120 drops per minute (8 cc.). With the more concentrated solutions and rapid infusion rates, under emergency conditions, the cardioscope has been used for quick detection of possible hyperkalemia. As a rule, the potassium salt, kept available in sterile 1 to 3 gram lots, is simply added to whatever infusion mixture seems otherwise most desirable, be it 5 or 10 per cent glucose, a protein hydrolysate or some other solution. Even when so large an amount as 467 mEq. of potassium was given in eighteen hours (43), the concentration of potassium in the infusate did not at any time exceed the figures above, for the amount of fluid injected during the period was 10 liters. Circumstances may arise, as in the presence of heart failure, in which it would be necessary to use a more concentrated solution and less water; it would then be wise to inject the solution at a slower rate.

There have been no personal experiences causing grief with this type of procedure over a period of five years, but there is no doubt that potassium salts should be used with every precaution. A case in point has come to our attention. A patient was clearly suffering with hypokalemia, and the need for potassium was urgent. An overzealous physician injected intravenously within a few minutes a dose of potassium salt which should have been given during an hour or more. While his colleague watched the cardioscope, the picture changed abruptly from that of low potassium to high potassium; and before injection could be stopped, the patient expired.

However, our recent tendencies have been to give larger, rather than smaller, doses. When hypokalemia has appeared in these debilitated types of patients, the receptivity of the cells for the injected potassium has appeared to be very great; and as shown in the preceding graphs, it has usually taken several days to "fill the stockpiles" before the serum potassium has returned to normal levels. In the face of any reduction of urinary volume, it is well to go slowly; and frequent recourse to cardiography or serum potassium determinations is in order.

A word as to the routine use of potassium salts in convalescence. A well-rounded average diet contains, of course, an abundance of potassium, 90-125 mEq. per day. If one can eat beef steak and potatoes, well and good, but in a weakened state after infections or operations, one is apt to turn against even such delectable modern rarities. Perhaps some of the long attested virtue of certain "home" and "folk" remedies lies in their content of potassium. When we were sick as children, our grandmothers would

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CHANGES IN CIRCULATING EOSINOPHILS IN WOMEN DURING THE MENSTRUAL CYCLE AND REPRODUCTION*

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VARIATIONS in the number of circulating eosinophils were noted as early as 1910 by Dunger (1) who first devised an accurate method for counting these cells. A marked rise in the number of eosinophils is associated with many pathologic conditions. However, a decrease in the eosinophil count has been regarded as of little importance. As an example, the recently published textbook on disorders of the blood by Whitby and Britton (2) attaches no significance to eosinophil counts under 400 per cubic millimeter. However, Schilling (3) described an eosinopenia in the course of acute infections and regarded the failure of the eosinophils to return to the circulating blood as a grave sign.

Renewed interest in circulating eosinophils followed the report of Dougherty and White (4), who demonstrated that pituitary adrenocorticotrophic hormone (ACTH) injected into rats produced an increase in the circulating neutrophils and a decrease in lymphocytes. They induced similar changes by the injection of 11-dehydroxy and 17-hydroxy corticosterone. De la Balze, *et al.* (5) found that in patients with Cushing's syndrome there was a leucocytosis and neutrophilia with a relative lymphopenia whereas in patients with Addison's disease there was a relative neutropenia and lymphocytosis.

Forsham and his associates (6) reported that ACTH produces a fall of circulating eosinophils in patients with normal adrenal cortical function. Patients with Addison's disease failed to show this response. These investigators observed that the fall in circulating eosinophils was approximately twice as great as the drop in lymphocytes and, since the difference in response between Addisonian and non-Addisonian individuals was clear-cut, the number of circulating eosinophils is the more sensitive indicator of adrenal cortical function. Thorn and his associates (7) reported that epinephrine reduced the circulating eosinophil count in normal individuals but failed to do so in patients with adrenal cortical failure.

The adrenal gland undoubtedly plays an important role in the repro-

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onset of contractions early in labor when the cervix was about 5 or 6 centimeters dilated, at the onset of the second stage, and immediately postpartum. Further blood samples were obtained on the first, third, fifth and ninth postpartum days.

RESULTS

Normal women.—Eosinophil counts were made in 100 carefully selected normal individuals. This group was used to establish normal values in women during their reproductive years. There were marked variations in the total white counts among the individuals of this group, but repeated counts in the same individual showed very consistent results. The white count varied from 4,750 to 12,500, within the accepted normal range. The mean percentage of eosinophils was 2.3 (Fig. 1).

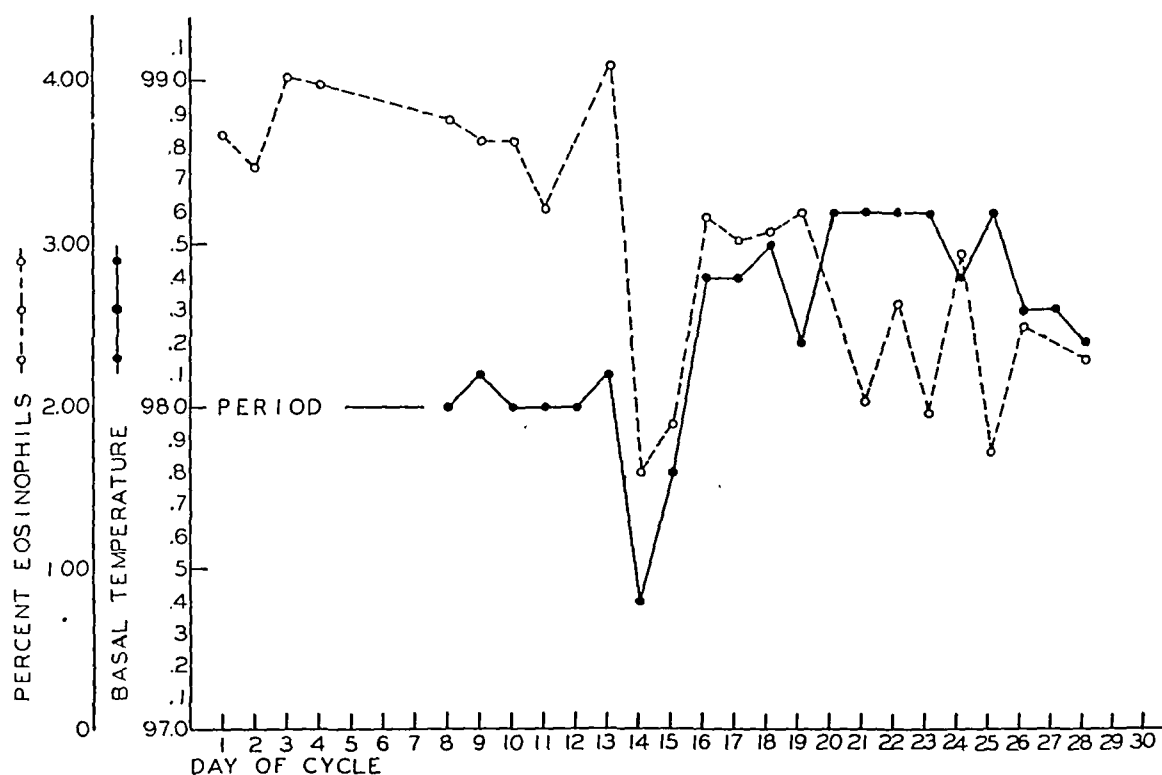


FIG. 2. Circulating eosinophil counts during a typical menstrual cycle. Note the drop in eosinophils about the time that the body basal temperature shifts from the low pre-ovulatory level to the elevated luteal level. There is a tendency for the count to be lower in the luteal phase.

The menstrual cycle.—Variations in circulating eosinophils were studied in 8 normal young women. The ovarian cycle was followed by means of basal body temperatures in order to determine the period of ovulation. Two constant findings were present in all these individuals: 1) a drop in the number of circulating eosinophils occurred consistently at the time of ovulation, as indicated by the rise in body temperature, and 2) the counts were lower during the luteal phase than during the follicle phase (Fig. 2).

ductive function. There is ample evidence that adrenal cortical activity is intimately associated with the pituitary and gonads. The demonstration of the relationship between adrenal cortical function and circulating eosinophils prompted this study.

METHOD

White blood cell and eosinophil counts were made from oxalated venous blood. The cells were counted immediately but if this was not feasible, they were stored in the icebox. The oxalate tubes were prepared by the method of Forsham, *et al.* (6), and a Levy counting chamber which has a depth of 0.2 mm. and a ruled area of 16 sq. mm. was used.

Serial determinations were made in as many patients as possible. The number of circulating eosinophils during labor was calculated before the

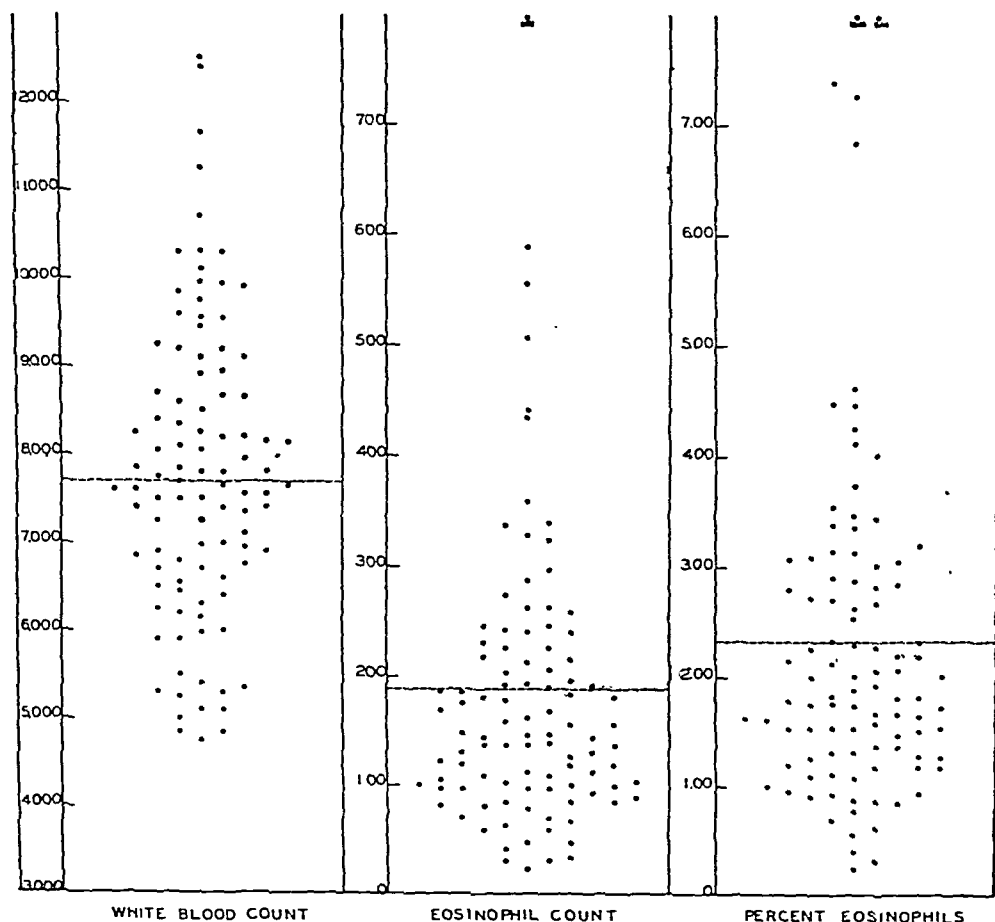


FIG. 1. The total white cell and circulating eosinophil counts in 100 normal young women. The eosinophils averaged 2.3 per cent of the total white count.

The leucocytosis of pregnancy has disappeared completely six weeks after delivery.

The most striking change in the number of circulating eosinophils occurs during labor, and consists of an eosinopenia. The first significant manifestation of this change is exhibited during the first stage. The eosinopenia becomes progressively more marked during the second and third stages of labor, reaching an almost total eosinopenia in the immediate postpartum period. The complete disappearance of circulating eosinophils during the

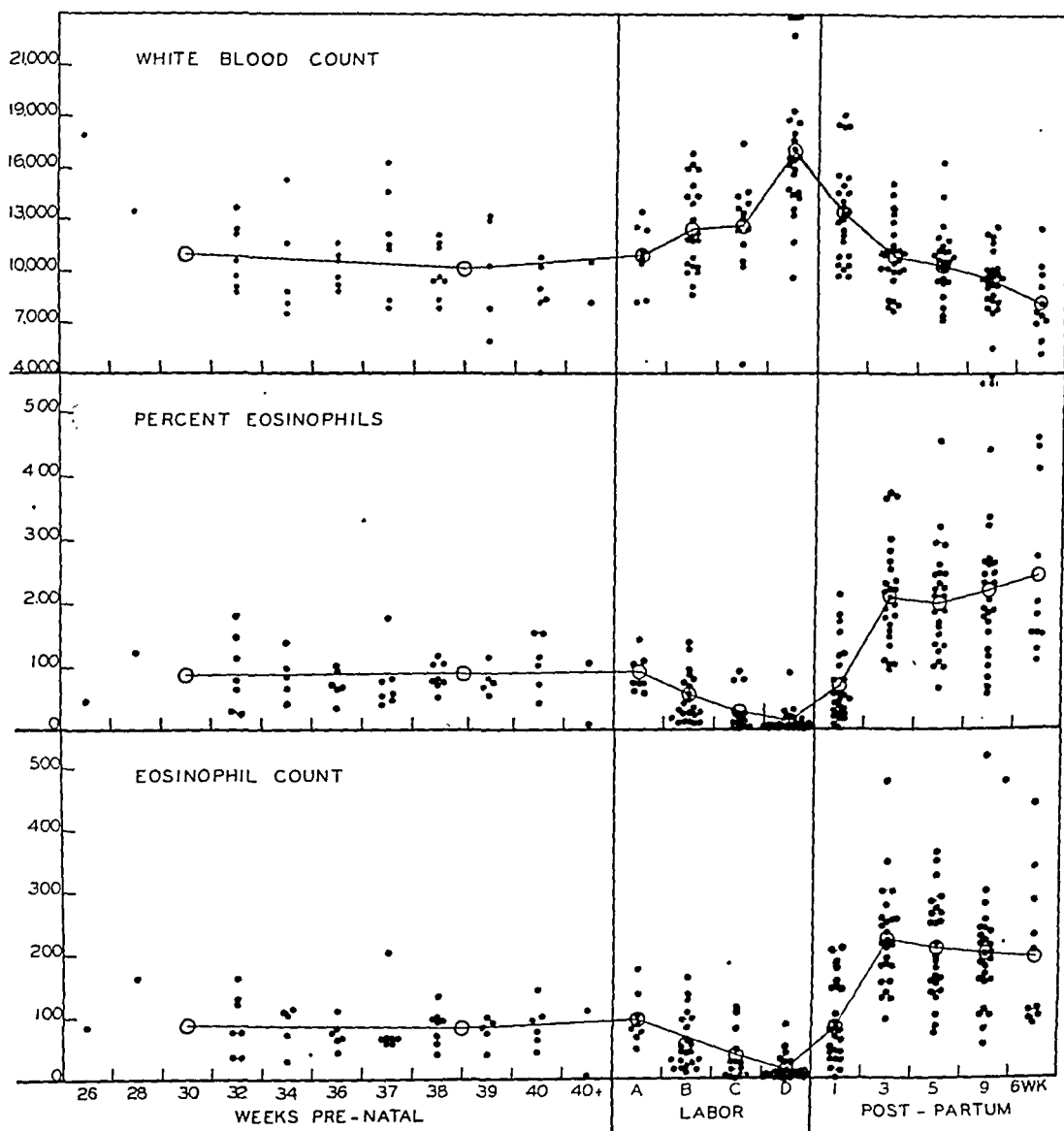


FIG. 4. The total white cell and circulating eosinophil counts of the group of patients followed during pregnancy, labor and the postpartum period. The most striking change is the eosinopenia which develops during labor, reaching a total eosinopenia at the end of labor in more than half the patients. The eosinophil count returns to the normal by the end of the third postpartum day.

Pregnant women.—Determinations were made on 300 normal patients at various periods in their gestations. There was no change in the circulating eosinophil count as the pregnancy advanced. Thus, the period of the gestation did not influence the count. However, a leucocytosis is present in pregnancy. The mean total white count is increased from an average of 7,700 in the nonpregnant individual to a mean of 9,750 in the gravid woman. There is also a relative eosinopenia, the decrease averaging 53

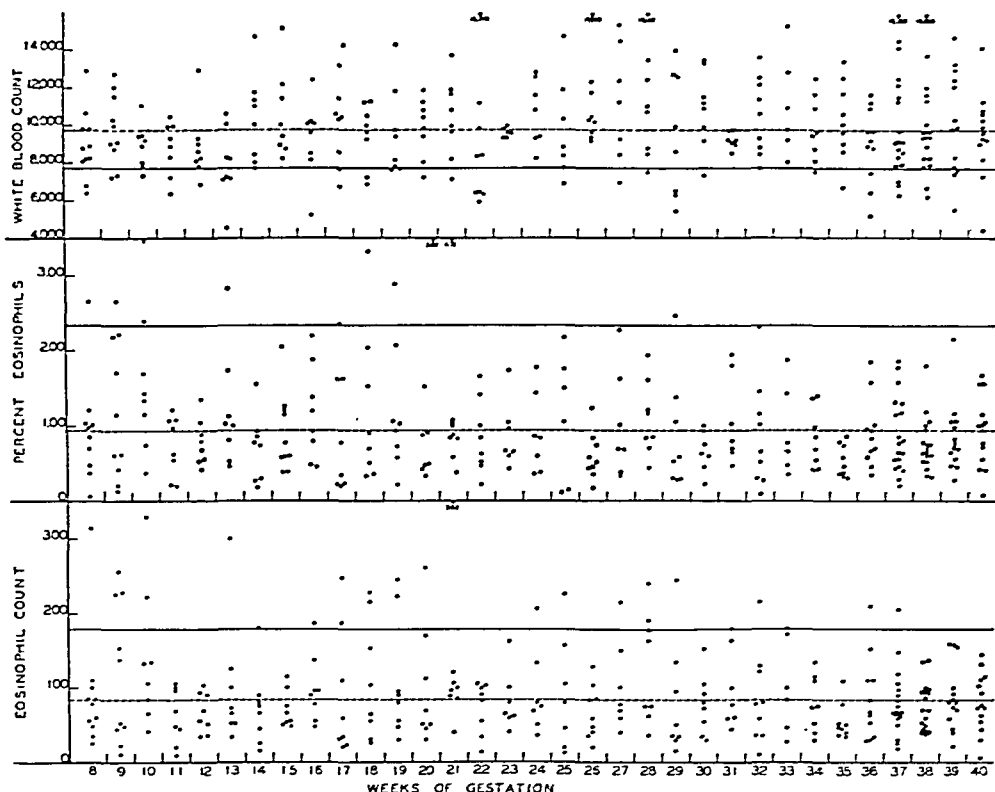


FIG. 3. Total white cell and circulating eosinophil counts in 300 pregnant women throughout gestation. The mean total white count was 9,750 compared with a mean of 7,700 in the nonpregnant control group. There is a relative eosinopenia in pregnancy averaging 0.9 per cent, compared with 2.3 per cent in the control group. However, the eosinophil count remains relatively constant in relation to the total white count, although there is an increasing leucocytosis as pregnancy progresses.

per cent when the nonpregnant and pregnant groups are compared. The calculated percentage of eosinophils decreased from 2.3 for the nonpregnant to 0.9 per cent for the pregnant group (Fig. 3).

The leucocytosis present during pregnancy increases rapidly during labor. The peak of this rising curve is reached in the immediate postpartum period, returning to preparturition levels by the ninth postpartum day.

labor. She was delivered of twins about six hours later. Counts were made one hour after the convulsion, immediately postpartum, and eight hours after her delivery. A total eosinopenia was present throughout this period. The eosinophils returned to the blood stream in appreciable numbers at

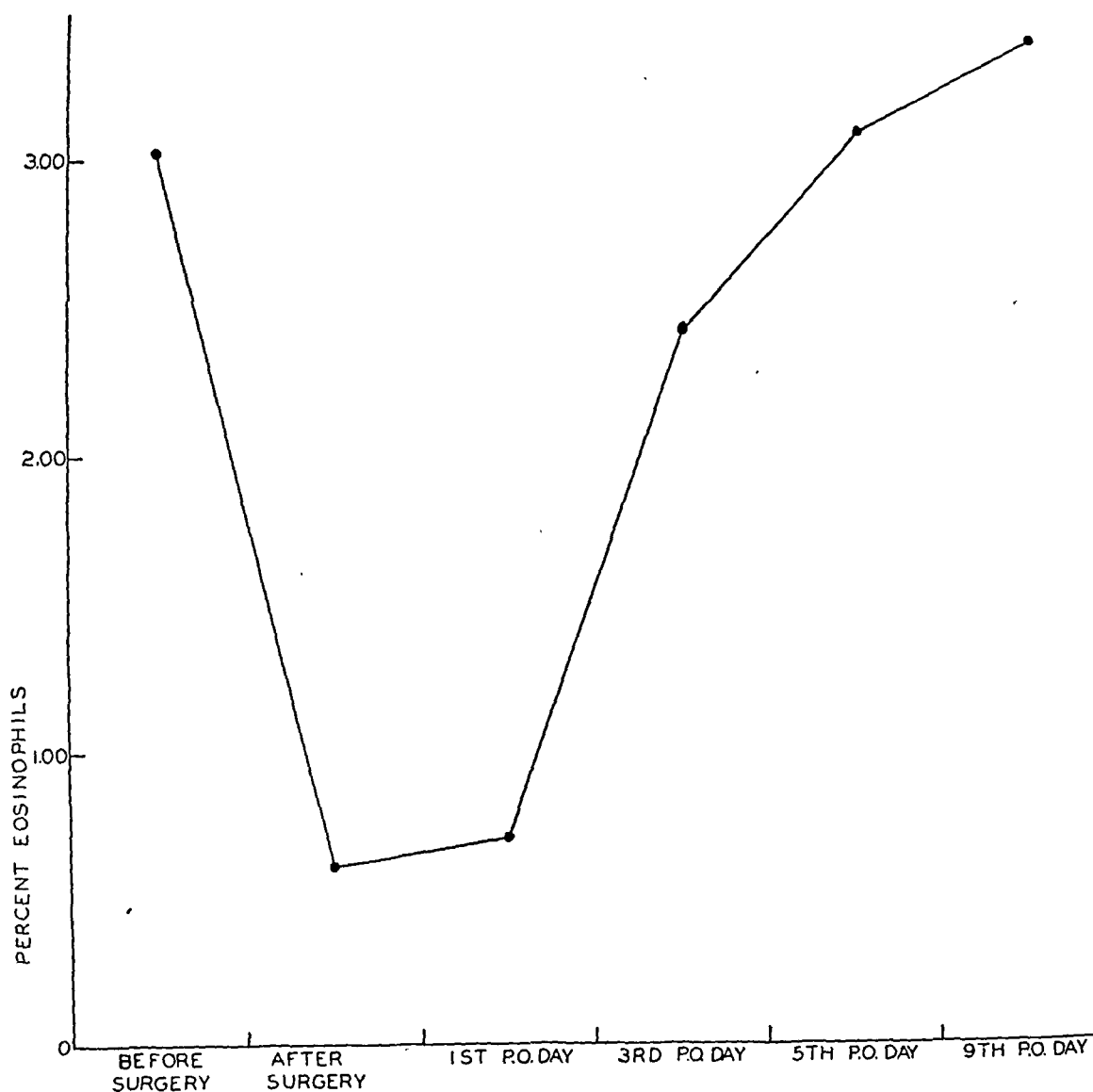


FIG. 6. Circulating eosinophil counts in 10 patients subjected to gynecologic surgical procedures. Note the marked eosinopenia which reached the low level three to six hours after surgery. The count did not return to normal for twenty-four hours.

the end of her first postpartum day, and normal values were reached by the third day.

Surgical operations unrelated to pregnancy.—In order to assess the role of the surgical phase of Cesarean section, 10 patients scheduled for gynecologic surgery, varying from a dilatation and curettage to complete hysterectomy, were studied. Counts were made before, during and after surgery, and on the first, third, fifth and ninth postoperative days.

final phase of parturition is exceedingly significant. The eosinophil count returns to the normal level by the end of the third postpartum day. The eosinophil response to labor is much more sensitive than the total white count, since the latter has not always returned to the normal nonpregnant level at the ninth postpartum day (Fig. 4).

Eight patients scheduled for elective Cesarean sections under local and general anesthesia were followed by means of eosinophil counts made on the first, third, fifth and ninth postoperative days. These women developed the same eosinophil response observed in the large group of women delivered naturally. The eosinopenia in women delivered abdominally

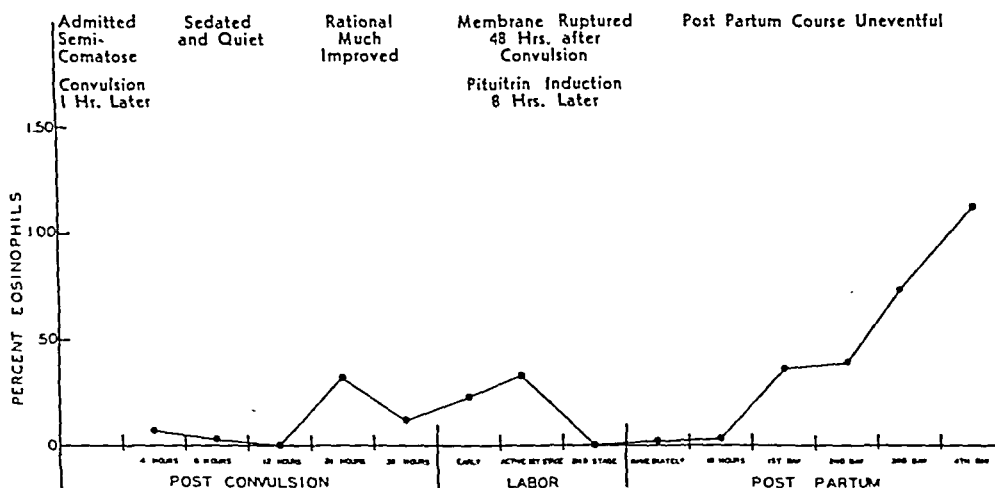


FIG. 5. Eosinophil counts in a patient with eclampsia. Note the almost total eosinopenia during and after the convulsive seizure and during the labor which followed. The return of the normal number of eosinophils to the circulation was delayed beyond the third postpartum day.

lasted a somewhat longer time than in the women delivered through the pelvis. The return to the normal values was postponed until the third postpartum day.

Eclampsia.—An opportunity was provided to study the eosinophil response in this important pregnancy complication in 2 patients. The first patient was not in labor when she had a convulsion. The circulating eosinophils disappeared completely during this seizure. She improved clinically during the next twenty-four hours, and the eosinophils returned to the circulation in small numbers. The induction of labor was followed by their complete disappearance a second time. The return of a normal number of circulating eosinophils following delivery was considerably delayed (Fig. 5).

The second patient had her convulsion during the active first stage of

DISCUSSION

The changes in the circulating eosinophils during the ovarian cycle is noteworthy. The number of cycles followed is too few to draw any accurate conclusions. However, the drop in circulating eosinophils has coincided with the period of ovulation consistently in all the cycles studied and appears significant. There is a tendency for the counts to be lower in the luteal phase than in the follicle phase. It is possible that ovulation itself may be a more profound physiologic stress than we have thought previously. The changes noted may indicate a sensitive gonadal pituitary adrenal balance.

Pregnancy is accompanied by an increase in the number of leucocytes and a decrease in circulating eosinophils. This relative eosinopenia is statistically significant. The stress of labor and delivery results in a rapid decrease of eosinophils reaching an absolute eosinopenia in many patients. These changes probably represent increased adrenal cortical activity, long continued during pregnancy, reaching a climax during parturition. Further studies are in progress which may demonstrate that these changes during reproduction have their origin in increased pituitary activity resulting in an increased production of ACTH, which, in turn, stimulates the adrenal cortex to secrete more cortical steroids.

Eclampsia is a complication of pregnancy that may have its origin in abnormal steroid metabolism. The absolute eosinopenia which developed during the convulsive seizures may represent sudden stress and compensatory adrenal cortical activity, perhaps mediated through the pituitary gland, or it may be an expression of abnormal glandular function. Pre-eclampsia and eclampsia deserve further study in this light.

Surgical intervention represents trauma, and the eosinopenia is the natural adrenal cortical response (Selye's "alarm reaction" (8)). The sensitiveness of the reaction is extremely interesting. Anesthesia seems to influence the eosinopenia very little, for similar responses were provoked in surgical procedures carried out under a variety of local and general anesthetic agents.

The role of the adrenal cortex during periods of stress has been known for many years. Numerous experimental and clinical observations indicate that the adrenal cortex secretes 11-oxysteroids as a protective mechanism during prolonged physical exertion, anoxia, exposure to cold or trauma, starvation and infection. These cortical steroids can be measured. Furthermore, adrenalectomized animals and patients with adrenal failure have a decreased tolerance to stress which can be increased by the administration of adrenal cortical extracts.

Thorn and his co-workers (7) demonstrated that the injection of ACTH

Preoperative counts were significantly higher than those observed in pregnancy. Following the operation, there occurred a profound drop of the eosinophil count reaching zero in half the patients. The lowest level of the eosinopenia was reached within three to six hours after the operation, and the extent of the drop varied with the magnitude of the surgery. The eosinopenia persisted for about twenty-four hours and the count did not return to the normal until the third postoperative day (Fig. 6).

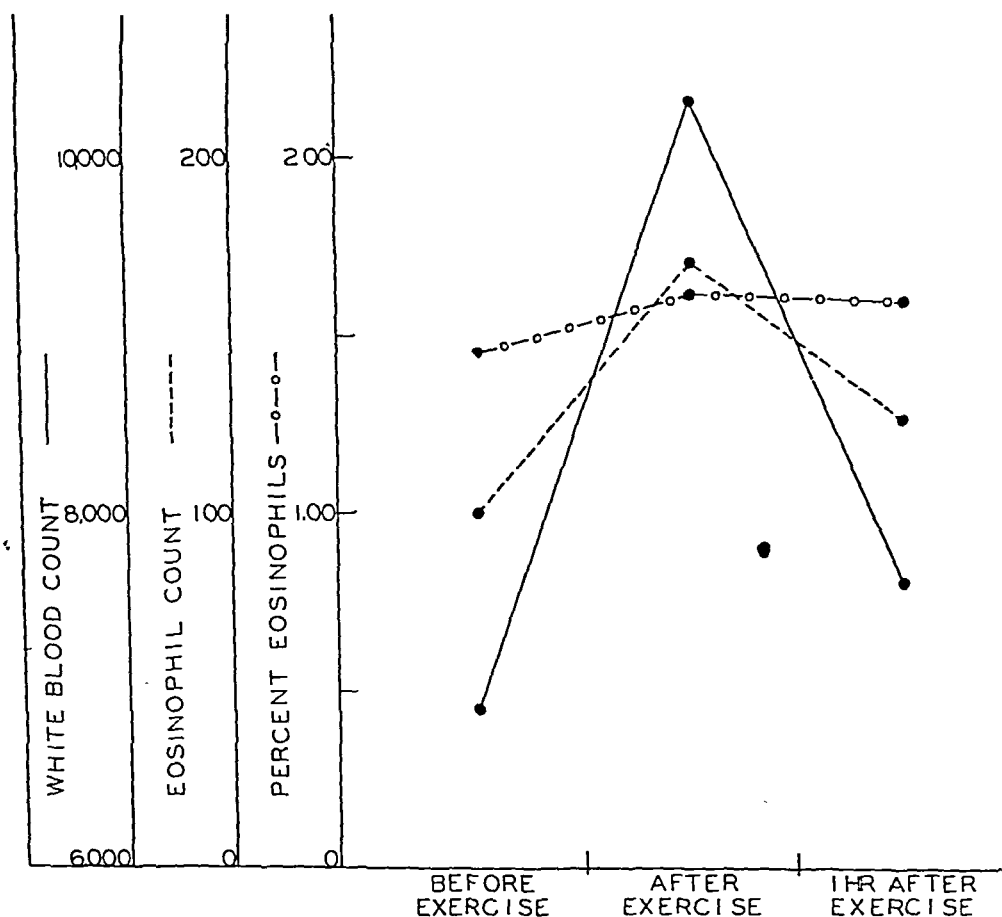


FIG. 7. The influence of moderate physical exertion on the white count and the circulating eosinophils. Moderate exercise results in a rise in the total number of white cells and eosinophils but the percentage of eosinophils remains constant.

Exercise.—The influence of physical exertion on the circulating eosinophils was demonstrated in 4 subjects. They were allowed to run around a square block at a brisk rate. Counts were made before, immediately after, and one hour after this exertion. There was a striking rise in both the total white cell count and the eosinophil count following exercise. The percentage of eosinophils remained relatively constant (Fig. 7).

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offered a method of ascertaining the function of the adrenal cortex. In the normal subject, the injection of ACTH is followed by a prompt fall in the number of circulating eosinophils and a rise in the acid-creatinine ratio in the urine. These effects are negligible in the presence of adrenal cortical failure. Recant, Forsham and Thorn (9) extended the usefulness of this observation by noting that the administration of epinephrine to normal animals produced an increase in adrenal cortical activity, but this response was absent in the hypophysectomized animal. This action presumably stems from the stimulation of pituitary function by the epinephrine and the subsequent release of ACTH, which in turn stimulates adrenal cortical activity. Thus, relatively simple methods are available not only for the detection of primary pituitary and adrenal disease, but they may be applicable in uncovering transient adrenal impairment as a result of surgical or obstetrical procedures.

These studies reveal that labor produces a period of physiologic stress which is easily combatted by normal adrenal function. Serious complications of pregnancy, such as eclampsia, produce more marked changes. It is possible that some of the previously unexplained states of shock in pregnancy and labor may well have their origin in transient pituitary or adrenal cortical failures. Their diagnosis and treatment should be facilitated by our new knowledge.

SUMMARY

The numbers of circulating eosinophils were determined in normal women during the various phases of the ovarian cycle, during pregnancy, delivery and the postpartum period, during and following Cesarean section and gynecologic operative procedures in eclampsia and following moderate physical exertion. A correlation of circulating eosinophils and the ovarian cycle was demonstrated. Pregnancy results in a leucocytosis and a relative eosinopenia. Labor is associated with an increasing eosinopenia so that delivery is often climaxed by a total absence of circulating eosinophils. Eclampsia and surgical procedures are characterized by a marked or absolute eosinopenia. The significance of these physiologic changes is discussed.

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more effective sublingually than orally on the basis of vaginal smear changes. They concluded that stilbestrol was twice as effective when given intramuscularly (0.5 mg. being equivalent to 1.0 mg. orally) in this "human assay." Shorr (9) reviewed the advantages of vaginal smear studies and made some comparisons of orally administered estrogens. He noted an equivalent response to ethinyl estradiol, 0.2 mg.; diethylstilbestrol, 2.0 mg.; and monomethylstilbestrol 4.0 mg., when these doses were given daily for an unstated interval. He also found that 2.0 mg. of stilbestrol orally was equivalent to 0.5 mg. estradiol benzoate intramuscularly. Allen (10) determined the minimal doses that would induce bleeding in castrate women. On the basis of this endometrial response, he found that an oral dose of ethinyl estradiol, 0.4 mg., was equivalent to 3.0 mg. of stilbestrol. This ratio of 1 to 7.5 compares favorably with Shorr's ratio of 1 to 10. Allen also determined that stilbestrol, 3.0 mg., orally, was equivalent to 1.0 mg. of estradiol benzoate intramuscularly. This 3:1 ratio approximates the 4 to 1 value of Shorr but differs from the 1.0 to 0.8 findings of Stoddard and Metzger.

Ryden (11) compared the estrogen effect in 8 castrate women, using weekly endometrial biopsies, and concluded that Dinestrol injected intramuscularly was 5.7 times as effective as when given orally. Dinestrol and estradiol benzoate were equivalent in potency when given intramuscularly.

The purpose of this study was to determine the minimal effective dose of various estrogens as revealed by changes in the vaginal smears of postmenopausal women.

MATERIAL AND METHODS

Estrogens available on the market as well as some special preparations were used. When given orally, they were administered daily for ten days, following the pattern set by Stoddard and Metzger. Intramuscular preparations were given as single injections. Stilbestrol tablets were purchased from the local pharmacy. Crystalline stilbestrol furnished by Smith-Dorsey was made up in sesame oil (5 mg./cc.) and so diluted that each test dose was given in 1 cc. of oil. Estrone (Theelin-In-Oil) was furnished by Parke, Davis & Co. in ampoules of 10,000 i.u. (Lot No. 3165625) for intramuscular use, and in gelatin capsules containing 5,000 i.u., for oral administration. The mixture of estrone and estriol (Theelin and Theelol, 0.5 mg. of each in oil per gelatin capsule), estriol (Theelol, 0.25 mg. per Kapseal, Lot No. F326F), and the mixture of natural estrogens (Menagen x1947 in tablets containing 10,000 i.u.) were also furnished by Parke, Davis & Co. An aqueous suspension of crystals and an oil solution of mixed estrogens (Estrogenic Substances) were furnished by Smith-Dorsey. Aqueous suspensions of large and small crystals (RB 134-44 and RB 134-43) of natural

THE USE OF THE HUMAN VAGINAL SMEAR IN THE ASSAY OF ESTROGENS*

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LABORATORY assay methods have proven inadequate to determine the relative clinical potency of the various estrogens. By rat assay, the apparent potency of estrone varies with the number of injections and the vehicle in which the hormone is injected. Laqueur (1) found the rat unit of estrone equivalent to 10 to 16 international units, but Rowe and Simond (2) obtained a value of only 3 or 4 international units. Estradiol benzoate is approximately ten times as potent as estrone when assayed in the rat, but only four times as potent (Laqueur) or of equal activity (Rowe and Simond (3)) when tested in the mouse. The outstanding example of misleading laboratory assay is the discrepancy between rat assays and clinical trials of the methyl ether of bis-dehyrdo-doisyndolic acid. In the rat, this compound when given subcutaneously is more active than estradiol, whereas, clinically it is practically inert (Segaloff (4, 5)).

Since 1941 many studies on the amounts of various estrogens necessary to relieve subjective menopausal symptoms have appeared, but relatively few authors have used objective criteria to compare different estrogens, or the same estrogen by different routes. Mack and Ale (6) by daily vaginal smears from a group of women demonstrated that estrone would restore glycogen to the vaginal epithelium. The smallest dose employed was 5 mg. There was no appreciable difference in effect, except that the oral dose produced a less sustained response. Stoddard and Metzger (7) studied vaginal smears taken twice weekly before, during and after ten-day treatment intervals. By intramuscular administration, estradiol benzoate was only twice as effective as estrone. Furthermore 0.4 mg. estradiol benzoate intramuscularly was equivalent to 0.5 mg. diethylstilbestrol given orally.

Castrodale, Loeffel and MacBryde (8) found that stilbestrol was no

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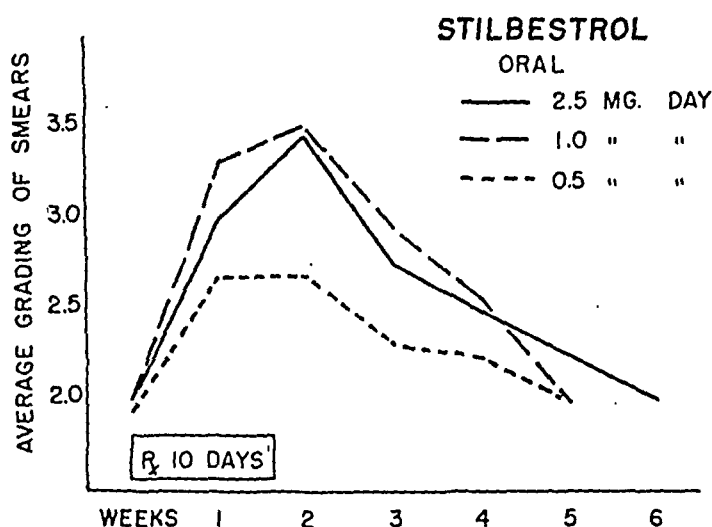


FIG. 1. Graphic representation of the vaginal smear changes induced by various doses of stilbestrol given orally for ten days. These observations were successive tests made on the same group of patients.

parison due to difference in duration of administration of the estrogen by the two routes. Castrodale, Loeffel and MacBryde found the potency of the oral dose to be only twice that of the intramuscular when daily doses were administered in each instance.

Estrone (Theelin-In-Oil) given orally to 16 women in doses of 2.0 mg. and 1.0 mg. daily produced equally marked changes in the average smear grading (Fig. 3). Again, as after stilbestrol, five or six weeks elapsed before

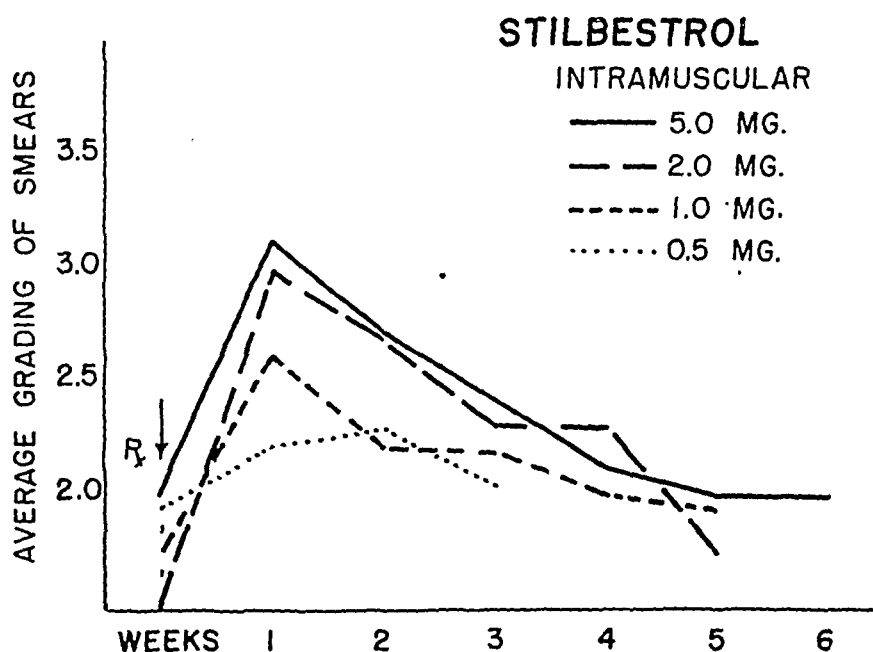


FIG. 2. Graphic representation of the vaginal smear changes induced by an intramuscular injection of stilbestrol in 1 cc. of oil. These observations were made on 12 patients remaining from the original group of 19 who were given stilbestrol orally.

estrogens and oily solutions of trimethyl estradiol acetate (Estrotate, Lot No. RB 11-1D), in concentrations of 0.33, 0.50, 1.0 and 1.66 mg./cc. were furnished by Lakeside Laboratories.

The subjects were postmenopausal women housed in the Iowa State Hospitals and were selected because they showed atrophic vaginal smears. Groups of at least 10 and usually 15 to 20 women were given the same dose of an estrogen and the degree and duration of vaginal responses were noted. The women were given a large "priming" dose of estrogen (5 mg. of stilbestrol or 4 mg. of a natural estrogen), and when the smears had regressed to their original stage, usually after four to six weeks, a test dose was given. When this test dose produced a definite response it was reduced one-half and the next trial made after the vaginal smears had regressed again. In this procedure, the large doses sufficed to "prime" the subject for the subsequent reduced dose. When a dose was reached which induced a very minor response or none at all, it was concluded that the minimal effective dose had been determined.

Smears were obtained weekly by inserting a moist cotton swab into the vagina. Then the swab was rolled over a slide and the smear allowed to dry in the air. These were examined as dry, unstained preparations under a low power objective.

Smears were recorded in four grades: *Grade 1*, an atrophic stage with only small basal epithelial cells and leukocytes. *Grade 2*, predominantly intermediate cells characterized by large, round cells with large, vesicular nuclei. These cells may contain considerable glycogen as demonstrated by the Mack iodine vapor stain method. *Grade 3*, early stages of cornification with many leukocytes. *Grade 4*, fully cornified smear with very few leukocytes.

EXPERIMENTAL

An oral dose of 2.5 mg. diethylstilbestrol per day for ten days increased the average grade of the vaginal smears of 22 women from 2 to 3.5 and then it regressed in the next three weeks (Fig. 1). One milligram orally per day produced very similar results but 0.5 mg. per day produced only half the rise in smear grading, and this was judged as a minimal dose.

Diethylstilbestrol was then administered intramuscularly to 19 women. A single injection of 5.0 mg. produced a marked response and it was five weeks before the vaginal smears returned to their initial stage (Fig. 2). A single dose of 2.0 mg. gave nearly as great a response, whereas 1.0 mg. produced definite but minor responses and 0.5 mg. was ineffective.

From these findings, it appears that a single intramuscular dose of 1.0 mg. of stilbestrol produces a response similar to that induced by 1.0 mg. orally per day for ten days. Possibly this ratio of 1:10 is not a fair com-

induce withdrawal bleeding. He found that the effective oral dose of estrone was ten times larger than that of stilbestrol (31.5 mg. per week for estrone as compared to 3.0 mg. per week for stilbestrol).

Estrone (Theelin-In-Oil) given intramuscularly produced marked changes in the vaginal smear when given in a single dose of 4 mg. or 2 mg. (Fig. 4). A dose of 1.0 mg. did not induce any distinct rise in vaginal cornification but the initial grading for the group was higher than usual. The test should be repeated at the 1.0 mg. dose level but it seems probable that it will be about the minimal effective dose, since the response to 1.0 mg. of stilbestrol is very similar to that of 2.0 mg. of estrone, whereas 0.5 mg. of stilbestrol was without effect.

Estriol (Theelol) given orally (1 mg. per day) induced no demonstrable

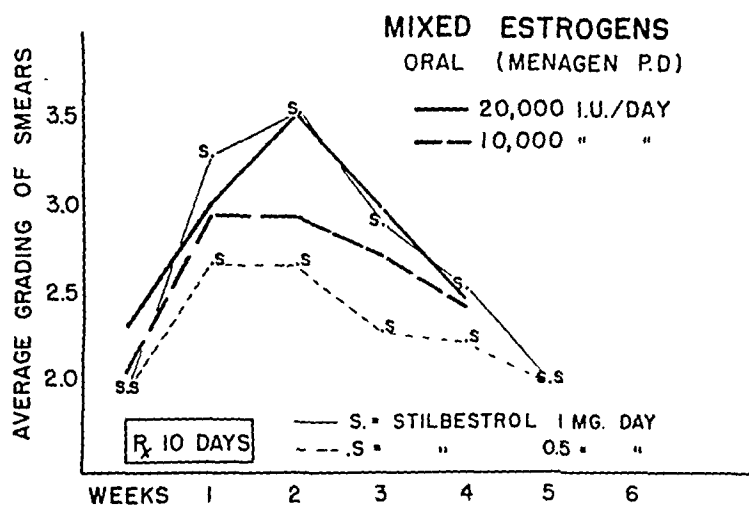


FIG. 5. Graphic representation of the vaginal smear changes resulting from the oral administration of natural estrogens (Menagen) for ten days. The curves showing the response to stilbestrol are transferred from Figure 1.

effect on the vaginal smear (Fig. 3). This ineffectiveness of estriol was further manifested by the failure of estriol to exert any additive effect over that obtained with estrone alone. This lack of effect of oral estriol is in marked contrast to the experimental findings in rats. Rowe and Simond found that it required 5 micrograms of estrone (15 subcutaneous rat units) to produce an effect when given orally, whereas only 3.0 micrograms of estriol (3 subcutaneous rat units) induced an estrous smear when given orally. On a rat-unit basis, estriol was five times as potent as estrone for oral use but on a weight basis (3.0 micrograms were equivalent to 5.0 micrograms) the advantage was more apparent than real. Even this slight advantage of oral potency of estriol in rats does not appear to have any clinical application, 1.0 mg. of estriol being ineffective, whereas 1.0 mg. of estrone produced a maximal response.

The mixture of natural estrogens occurring in pregnant mare's urine

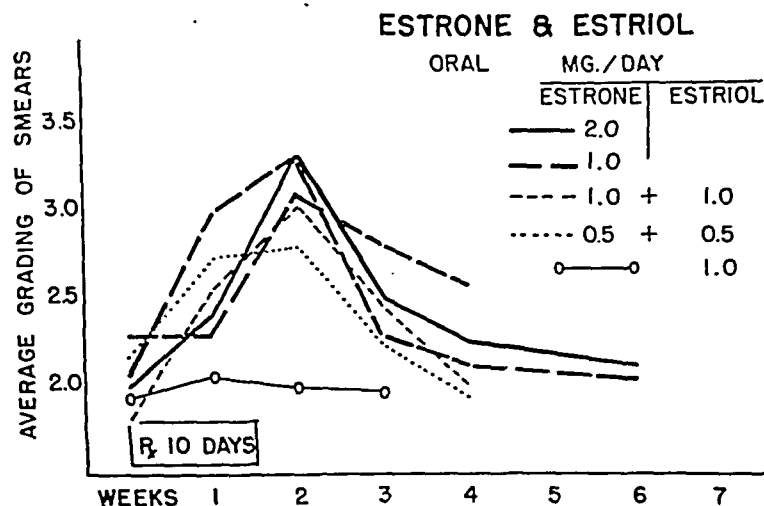


FIG. 3. Graphic representation of the vaginal smear changes resulting from the oral administration of estrone, estriol, and estrone with estriol for ten days (Theelin and Theelol-Parke, Davis & Co.). The two tests with the 1 mg. dose of estrone are plotted for comparison. At these dose levels, estriol was ineffective by itself and had no additive effective when given together with estrone.

the smears regressed to their former grades. When 0.5 mg. was given, the response was neither as great nor as prolonged, so that 0.5 mg. appeared to be about the minimal effective dose. These findings confirm those of Mack and Ale that estrone is effective when given orally. Furthermore, estrone appears to be as effective as stilbestrol, milligram for milligram, when given orally. These findings on changes in vaginal smears are in marked contrast to those of Allen, based on minimal doses required to

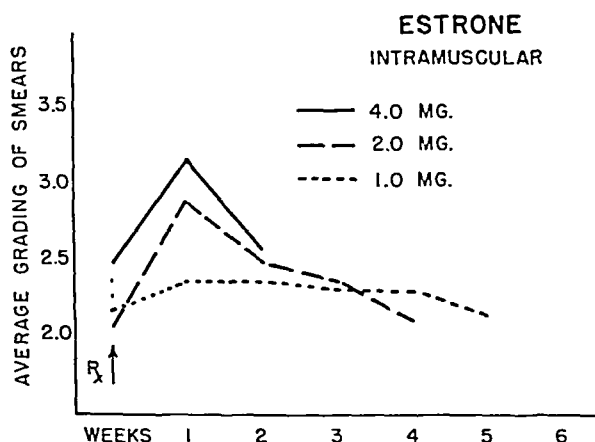


FIG. 4. Graphic representation of the vaginal smear changes following an intramuscular injection of estrone (Theelin-In-Oil).

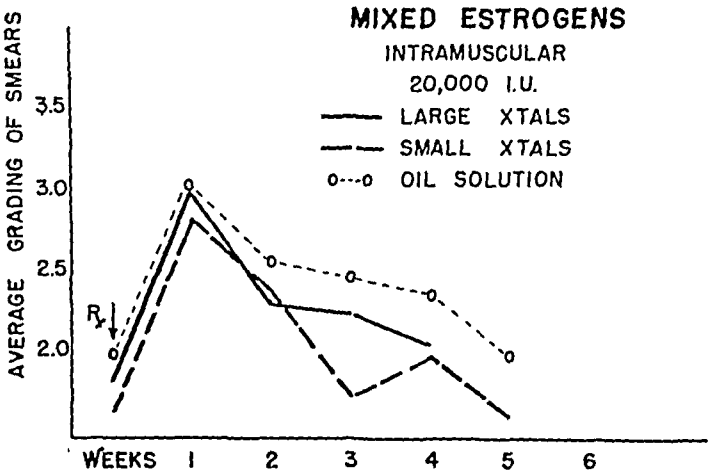


FIG. 7. Graphic representation of the vaginal smear changes induced by an intramuscular injection of aqueous suspensions of large and small crystals of natural estrogens (Lakeside Laboratories). The curve showing response to an oil solution is transferred from Figure 6.

Wisconsin. It seems probable that even the large crystals were so small that the relative difference in size was not sufficient to change their rate of absorption appreciably since their effect was not prolonged over that of an oily solution. Both sizes would have been graded as fine crystals by Miescher, Gasche, and Frey (12) who list less than 50 microns as fine crystals, 50 to 150 microns as medium and 150 to 250 microns as coarse. They also found that flat platelet crystals are more quickly absorbed due to their relatively greater surface area per unit of volume.

The most potent estrogen encountered in this study was trimethyl estradiol acetate (Estrotate-Lakeside). The dose was reduced from an initial 3.3 mg. to 0.5 mg. without any marked decrease in response (Fig. 8): A dose as low as 0.16 mg. still produced an effect greater than that following

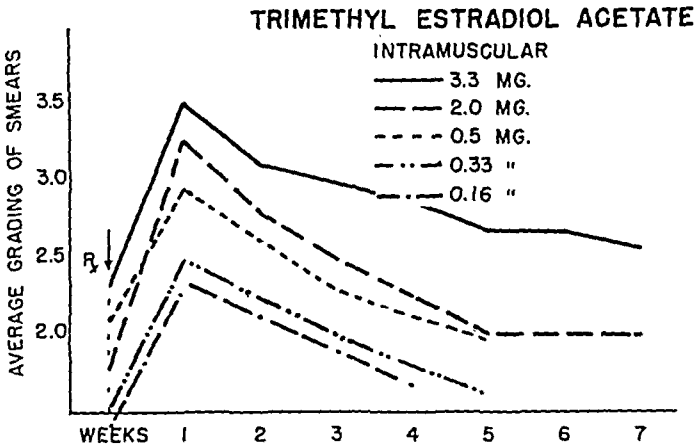


FIG. 8. Graphic representation of the vaginal smear changes following an injection of trimethyl estradiol acetate (Estrotate-Lakeside).

(approximately 90 per cent estrone) was tried in several forms by oral and intramuscular routes. An oral preparation (Menagen) was given at two dose levels. The change in vaginal smear grading in 11 women given 2 capsules (20,000 i.u.) daily was similar to that induced by 0.5 mg. of stilbestrol (Fig. 5). In this instance, it is not possible to make a weight comparison. However, it has been shown that stilbestrol subcutaneously by rat assay is about 2.5 times as potent as estrone (25,000 i.u. as compared to 10,000 i.u. per mg.). On this basis, the human response to oral administration of these two substances shows a good correlation with their respective estrogenic potencies in international-unit equivalents.

The natural mixed estrogens were given intramuscularly as aqueous suspensions of crystals and as oily solutions. An aqueous suspension of crystals (Smith-Dorsey) was given at two dose levels, 40,000 i.u. and 20,000

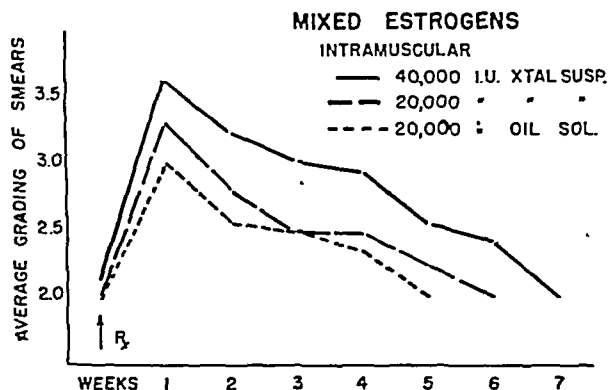


FIG. 6. Graphic representation of the vaginal smear changes induced by an intramuscular injection of natural estrogens (Smith-Dorsey) as aqueous suspensions of crystals and in oily solution.

i.u. The larger dose produced a somewhat greater and more prolonged response (Fig. 6) but there is little appreciable difference in degree or duration of response from 20,000 i.u. as crystals, compared to the same dose in an oily solution (Smith-Dorsey). By comparison, the responses to 2.0 mg. (20,000 i.u.) of estrone and 1.0 mg. of stilbestrol (25,000 i.u.) were almost identical to that of 20,000 i.u. of natural mixed estrogens in oil. Here again the effectiveness of the natural estrogens and stilbestrol were comparable on the basis of international-unit equivalents.

Two sizes of crystals were used to determine whether larger crystals might prolong the effectiveness. Aqueous suspensions of large crystals and small crystals were prepared by Lakeside Laboratories. There was no appreciable difference in effect when given in 20,000 i.u. doses (Fig. 7). The average measurements of these crystals in microns (large 10.62×7.7 , small 8.7×4.0) were determined by Prof. V. W. Meloche, University of

1. Estrone given orally in daily doses for ten days is as effective as stilbestrol.
2. Estrone given intramuscularly in a single dose is about one-half as potent as stilbestrol.
3. Estriol in the oral dosage used (1 mg. per day) had no demonstrable estrogenic effect, whereas estrone was effective.
4. Natural estrogens (approximately 90 per cent estrone) are effective orally and compare favorably with stilbestrol on an international-unit basis.
5. Suspensions of crystalline estrogens do not have any apparent depot effect when the crystal dimensions are no greater than 10 microns.
6. Estradiol (trimethyl acetate) produced maximal response in doses of 0.5 mg. intramuscularly and there was a minimal response following 0.16 mg. intramuscularly.

Addendum

Since this article was written, assays of trimethyl estradiol acetate by the oral method have demonstrated maximal vaginal response in doses of 5.0 mg., 2.0 mg., and 1.0 mg. daily for ten days, and considerable activity in a dose of 0.5 mg. daily.

Acknowledgments

We are indebted to Drs. Soucek and Ristine of the Mount Pleasant State Hospital for making patients available for this study.

This study was aided by grants from Parke, Davis & Company, Lakeside Laboratories, and Smith-Dorsey.

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0.5 mg. of stilbestrol. Thus by intramuscular injection, the trimethyl ester of estradiol acetate appears to be at least three times as potent as stilbestrol in stimulating the vaginal epithelium of women.

An unsuccessful attempt was made to study estrogen withdrawal bleeding. The subjects of this study were mental patients and their history of such bleeding was unreliable. The comments of the attendants were likewise found to be unreliable. Though on many occasions blood was found on the vaginal smear, it was felt that the single weekly contact with these women did not give satisfactory data as to the incidence, amount, or duration of bleeding.

Bleeding did occur in some members of each group when large doses were used and it is our impression that this sign was more frequent when oral medication was employed. Further studies on more cooperative subjects will be necessary to determine the uterine threshold, or withdrawal-bleeding requirements of these estrogens.

DISCUSSION

The reasons for the apparent differences in potency of estrogens in different species probably will be found in metabolic studies. The mouse seems unable to split the benzoate off the estradiol ester as readily as the rat. The ability of the rat liver to inactivate estrogens may be greater than that of the human (13).

Hooker, Drill and Pfeiffer (14) found that pellets of estrogen implanted in the spleen of the monkey were as effective as those implanted subcutaneously. This is in marked contrast to the inactivation of estrogens injected into the spleen of rats (Segaloff). Clinical studies designed to test the relative effectiveness of estrone and of estradiol when given orally or intramuscularly in single or in divided doses are necessary before the effect of the liver can be assessed. The fact that estriol is a form of estrogen peculiar to the human (it is excreted in the urine of pregnant women and has not been found in other species) makes it possible to rationalize species difference in response to this substance. Estriol should be tried in doses of 5.0 or possibly 10.0 mg. per day to learn whether this excreted substance has any estrogenic potency in women. The estrogenic action of the synthetic nonsteroid substances will be an enigma until their metabolic breakdown is known.

SUMMARY

The species variation in response to various estrogens is so great that clinical evaluation of these substances is dependent upon trial in human subjects. When compared in postmenopausal women on the basis of the vaginal smear response it was found that:

PROGESTERONE: A COMPARISON OF INTRA-MUSCULAR, ORAL AND SUBLINGUAL ROUTES OF ADMINISTRATION*

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THE widespread use of progesterone in combination with estrogen in the cyclic steroid therapy of menstrual irregularities, together with its empirical use in the treatment of threatened and habitual abortion, makes urgent the need for effective progestational hormones which may be administered other than by injection.

It is generally believed that progesterone itself is inactive when administered by the oral route to human patients. This belief appears to be based on several studies in animals. A search of the literature has failed to disclose that the oral efficacy of progesterone in human patients has ever actually been adequately studied. It was apparently on the basis of the animal studies that anhydrohydroxyprogesterone (Pranone, Progestoral, Lutocylol) was synthesized and is now exclusively used as the only effective nonparenteral type of progesterone therapy.

In view of this, it was decided to investigate the sublingual and oral administration of progesterone itself in human patients. Actually the sublingual administration to human subjects of free progesterone has already been studied by Greenblatt (1). In 21 amenorrheic women the dose necessary to cause withdrawal bleeding was found to be from 125 to 150 mg. of progesterone. In the same study Greenblatt found the effective sublingual dose of anhydrohydroxyprogesterone to be 100 to 125 mg.—not a very striking difference between the two compounds.

METHODS

Patients with secondary amenorrhea associated with a persistent proliferative-phase endometrium were test subjects for this study. They varied in age between 19 and 27 years.

A type of Corner-Allen progesterone test used for assaying progesterone in animals, was employed with these patients. They were primed with estrogen (4,000 i.u. Urestrin¹ per day orally) for twenty days. On the last five days of the estrogen treatment they were then given the progesterone

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¹ Mixed natural estrogen (Upjohn).

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five days and of these, 2 had very scanty bleeding lasting less than an hour. Endometrial biopsy specimens taken twenty-four hours after completing treatment showed early progestational changes in 1 and no effect in 6 of the treatment cycles. There were 9 cycles in which 80 mg. daily in divided doses was given for five days and 1 of these was followed by four hours of uterine bleeding, not sufficient to require a vulva pad. Biopsy specimens taken within twenty-four hours on 4, and within forty-eight hours on 5, showed transitional endometrium in 5 and full progestational effect in 4.

TABLE 1. SUBLINGUAL PROGESTERONE THERAPY IN PATIENTS PRIMED WITH ESTROGEN (URESTRIN), 4,000 I.U. ORALLY PER DAY FOR 20 DAYS.

Dose of progesterone per day for last 5 days of estrogen treatment (mg.)	No. of cycles	Withdrawal bleeding	Endometrial biopsy
50	7	2 cycles, scanty bleeding	1 cycle, early progestational 6 cycles, no effect
	9	8 cycles, normal amount and duration 1 cycle, 4 hours' duration, scanty	5 cycles, transitional endometrium 4 cycles, full progestational effect
90	6	5 cycles, normal amount and duration 1 cycle, 8-hour period	1 cycle, full secretory effect 5 cycles, progestational effect

There were 6 cycles in which 90 mg. daily in divided doses was given for five days and in 1 of these a bleeding episode lasting about eight hours occurred. Endometrial biopsy on the day after completing the five-day treatment course was compared with the biopsy prior to the onset of treatment in the patient who had the bleeding response, and it was found that the endometrium was converted from a persistent proliferative type into a well developed secretory type. The other 5 biopsies showed evidence of progestational effect.

There was 1 patient (M2182), aged 31, who had a secondary amenorrhea of eighteen months' duration. During the first year of amenorrhea, cyclic uterine bleeding was induced with stilbestrol-progesterone, stilbestrol 5 mg. daily for twenty days and progesterone subcutaneously in a dose of 10 mg. on the fifteenth, twentieth and twenty-fifth days of the cycle. This treatment was discontinued and the patient lapsed into a period of six months' amenorrhea. She was then given Urestrin capsules (1 daily for

preparation. The progestational effect was determined by the amount of withdrawal bleeding and by endometrial biopsy.

RESULTS

Intramuscular progesterone

Prior to initiating this study or at some time during the study, it was shown that each of these 18 patients was capable of responding to intramuscular progesterone by the development of a progestational endometrium. The degree of endometrial response was variable, some responding with a well defined secretory endometrium to 5 mg. of progesterone daily for five days, whereas one patient required a minimum of 10 mg. daily for nine days to produce the slightest endometrial response. A fair to good secretory response invariably followed a dose of 10 mg. daily for five days when preceded by oral estrogen over a period of fifteen to twenty days. In all cases the progestational response was improved by the preliminary administration of estrogen.

Withdrawal bleeding occurred in every case primed with estrogen followed by five days of progesterone administration, but only 4 of 9 patients tested had withdrawal bleeding after progesterone alone.

Daily subcutaneous or intramuscular administration of progesterone in a dose of 10 mg. daily for five days usually induces progestational changes on the sixth day from the onset of treatment. Larger doses than 10 mg. do not seem to accelerate nor potentiate the progestational response and a dose less than 10 mg. per day for five days is followed by variations in the degree of progestational changes. With the knowledge that 10 mg. of progesterone intramuscularly daily for five days is approximately the minimal effective dose when given after priming with estrogen, it was adopted as the base line against which to project the results obtained from oral and sublingual administration.

Sublingual progesterone

Using patients from the preceding group after a rest period of at least three weeks since receiving intramuscular progesterone and a minimum of seven weeks of amenorrhea, there were 12 patients with secondary amenorrhea or oligomenorrhea of a functional nature treated by sublingual progesterone alone (Table 1). The hormone was administered to these patients in doses varying from 5 to 8 linguets daily for five days. Each linguet contained 10 mg. of pure progesterone, and the total of 50 to 80 mg. was given in divided doses three times daily. None of these patients had uterine bleeding within seventy-two hours after completing five days of sublingual progesterone therapy. There were 5 patients studied in 7 cycles of treatment who received the hormone in a dose of 50 mg. daily in divided doses for

conjunction with oral estrogen in the form of Urestrin, it was decided for purposes of comparison to start these patients on 80 mg. of anhydrohydroxyprogesterone in a divided dose daily for five days, in conjunction with Urestrin. This was done, endometrial biopsy specimens being taken before treatment and on the day following the completion of the five-day treatment. There was withdrawal bleeding in 8 patients, but a study of the endometriums showed no evidence of stimulation. Six of these patients were then studied during the oral administration of Urestrin daily for twenty days and 130 mg. of anhydrohydroxyprogesterone daily for five days on the last five days of Urestrin administration. Withdrawal bleeding occurred in 5, free bleeding in 4. Two patients who showed the maximum degree of uterine bleeding had a well developed secretory phase endometrium; lesser progestational effect could be seen in the other tissues studied. Three of these patients were then subjected to the same treatment schedule except that the anhydrohydroxyprogesterone dose was increased to 250 mg. per day for five days. Endometrial response was excellent. It was concluded therefore that 80 mg. of sublingual progesterone was approximately equivalent to 130 mg. of oral pregneninolone when used in conjunction with oral estrogen therapy for the induction of uterine bleeding in amenorrheic patients. Six patients with secondary amenorrhea varying in duration for eight to twenty-four weeks were treated by the oral administration of 150 mg. of anhydrohydroxyprogesterone with the simultaneous administration of estrogen. Uterine bleeding occurred in 5 of these patients, a transitional type of endometrium was found in 1, and a normal progestational endometrium in 4.

There was 1 patient with amenorrhea of twenty months' duration in whom withdrawal bleeding had previously been produced by other treatment. She was given anhydrohydroxyprogesterone in a dose of 80 mg. in a divided dose daily for five days with no bleeding and no endometrial response. She was then given the same drug orally in a dose of 110 mg. daily, with no response. A third course of treatment with 140 mg. daily for five days was not followed by bleeding, but there was evidence of progestational activity on endometrial biopsy.

Oral progesterone

A comparison of sublingual progesterone tablets placed in the oral cavity until dissolved was made with the same tablet swallowed (Table 3). Nine patients from the original group were available for study and 6 of them cooperated through the two treatment cycles. Progesterone was swallowed in a divided dose of 80 mg. daily for five days in 6 patients without previous priming with estrogen. There was no withdrawal bleeding, but endometrial biopsy revealed a transitional type in 3 and a fair progestational-

twenty days) and progesterone linguets (100 mg. daily in a divided dose on the last five days of Urestrin administration). She had free bleeding three days after completing this twenty-day treatment and endometrial biopsy revealed a well developed secretory phase with marked vascularity and gland lumens filled with secretion. The same patient was treated during the succeeding month with progesterone linguets alone. Linguets in a dose of 50 mg. daily were administered in a divided dose for five days; withdrawal bleeding did not occur and the endometrium was of the proliferative type.

There were 2 other patients with amenorrhea of 6 and 8 months' duration respectively. Treatment with Urestrin capsules (1 daily for twenty days) and progesterone linguets (50 mg. daily for the last five days of Urestrin administration in a divided dose) was followed by withdrawal bleeding in both of these patients. Their endometriums were proliferative in type.

TABLE 2. ORAL ANHYDROHYDROXYPROGESTERONE THERAPY IN PATIENTS PRIMED WITH ESTROGEN (URESTRIN), 4,000 I.U. ORALLY PER DAY FOR 20 DAYS.

Dose of anhydrohydroxy progesterone per day for last 5 days of estrogen treatment (mg.)	No. of cycles	Withdrawal bleeding	Endometrial biopsy
80	8	Occurred in 8 cycles	No evidence of progestational effect
130	6	Withdrawal bleeding in 5 No bleeding in 1	2, good progestational effect 4, moderate progestational effect

When these same patients were given progesterone linguets alone, no bleeding occurred and no endometrial response could be seen. They were then treated by Urestrin daily for twenty days and progesterone linguets 140 mg. daily in a divided dose on the last five days of estrogen treatment. Withdrawal bleeding occurred in both cases from a fully developed progestational endometrium.

Oral anhydrohydroxyprogesterone

The same 14 patients from the original group with secondary amenorrhea and oligomenorrhea who were available for comparative study with orally administered anhydrohydroxyprogesterone were used (Table 2). Since it had already been shown that the minimum effective dose of the hormone sublingually was approximately 80 mg. per day when administered in

further studies are now being conducted to confirm this preliminary report. These will provide a basis for a subsequent paper.

SUMMARY

1. Progesterone administered intramuscularly will consistently induce a progestational endometrium in the estrogen-primed woman after a dose of 10.0 mg. daily for five days.

2. Progesterone administered sublingually is effective in producing a progestational effect on the endometrium when given in a dosage of 80 mg. per day for five days.

3. Progesterone administered orally will produce a moderate progestational effect when given in a dose of 80 mg. daily for five days.

4. Anhydrohydroxyprogesterone administered orally produces a progestational effect on the endometrium in about 50 per cent of estrogen-primed patients who receive 80.0 mg. daily for five days.

5. Preliminary administration of estrogen is necessary for the full progesterone effect, whether the progesterone be administered intramuscularly, sublingually, or orally.

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TABLE 3. ORAL PROGESTERONE. (NO PRIMING WITH ESTROGEN)

Dose	No. of cycles	Withdrawal bleeding	Endometrial biopsy
80 mg. per day for 5 days (Progesterone)	6	No bleeding	3 cycles, transitional endometrium 3 cycles, fair progestational effect
80 mg. per day for 5 days (Anhydrohydroxy progesterone)	9	—	No progestational response

al type in 3. The same 9 patients were then given anhydrohydroxyprogesterone in a dose of 80 mg. daily; there was no endometrial response evident in any biopsy specimen studied. It had previously been shown in 5 of this group that a dose of 130 mg. of anhydrohydroxyprogesterone was the minimal effective dose for inducing progestational endometrium.

DISCUSSION

The studies on the effectiveness of the sublingual administration of progesterone to human patients reported here confirm and extend those of Greenblatt (1,2). In addition to the withdrawal bleeding employed by Greenblatt, another objective criterion, endometrial biopsy, was used. Since the withdrawal bleeding in these studies could have been on the basis of estrogen withdrawal, it is felt that the progestational effect observed in the endometrial biopsies is of greater significance.

The observation of greatest importance in this investigation is that the results indicate that progesterone itself is active when administered by the oral (swallowed) route. Furthermore, progesterone itself appears to be more active orally than anhydrohydroxyprogesterone. This is *completely contrary* to what has been believed to be true for man. As far as animal studies are concerned, the reports to date are controversial. Thus, Miescher and Gasche (2) found progesterone to be only one-sixtieth as active in rabbits as anhydrohydroxyprogesterone when administered orally. Soderwall (3), on the other hand, reported that progesterone was more active orally in inducing sexual receptivity in spayed female guinea pigs. One mg. of progesterone orally induced heat in 84.6 per cent of the animals, whereas 1.0 mg. of anhydrohydroxyprogesterone orally induced heat in only 62.5 per cent.

In view of the fact that our observation that progesterone is more active orally than anhydrohydroxyprogesterone is contrary to the accepted belief,

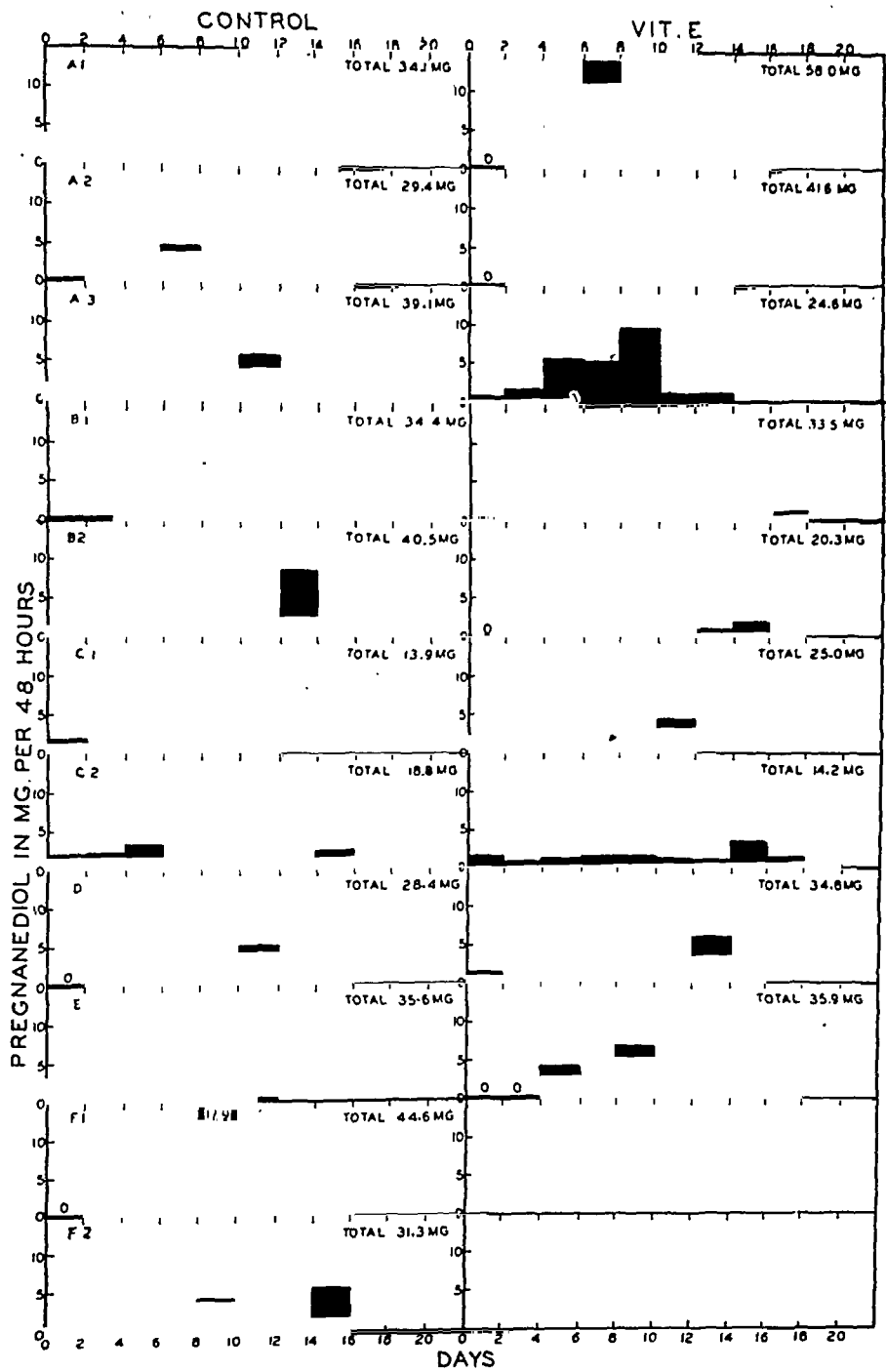


FIG. 1. The milligrams of pregnanediol excreted in the urine by normal individuals during 11 untreated menstrual cycles are compared with the amounts of pregnanediol excreted during the following menstrual cycles by the same individuals when alpha-tocopherol was administered. Pregnanediol is expressed as milligrams excreted per 48 hours, day 1 being the estimated day of ovulation.

THE EFFECT OF ALPHA-TOCOPHEROL ADMINISTRATION ON PREGNANEDIOL EXCRETION*

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VITAMIN E (alpha-tocopherol) has been widely used clinically in the treatment of disorders of reproduction since shortly after its discovery by Evans and Bishop (1) in 1922. It has been clearly demonstrated by animal experimentation that a vitamin E deficiency is associated with sterility in the male and resorption of embryos in the female rat. Any rationale for vitamin E therapy in related disorders of the human, however, has been lacking. The concept of a human diet deficient in vitamin E is difficult to accept and until recently no reliable, simple method was available for determining the vitamin E status of an individual.

Winkler (2, 3) and Winkler and Bach (4) have reported that the administration of vitamin E increases the ovarian and pituitary function in women. The increased function has been demonstrated by measuring the cyclic pregnanediol, estrogen and F.S.H. excretion of subjects before and during alpha-tocopherol administration. Stohler and Kaiser (5) in 1941 presented some experimental evidence for a similar effect in the rat. Such a finding is theoretically attractive, especially from the standpoint of estrogen and progesterone metabolism. If the anti-oxidant properties of vitamin E protect these unsaturated sterols from rapid oxidative destruction, the result might be an increase in the activity of these hormones. A sound basis for vitamin E therapy in some cases of sterility and habitual abortion would thus be established.

As only six feeding experiments with concomitant hormone excretion curves have been reported, it was deemed advisable to attempt to extend this work. Only the relationship of vitamin E administration to corpus luteum function has been investigated in the present study. Three normal laboratory workers volunteered for the experiment and seven feeding experiments were run on these individuals, all of whom were having regular menstrual cycles. Two additional feeding experiments were run on patients complaining of sterility but having regular ovulatory cycles as determined by endometrial biopsy. Two additional cycles on a sixth subject have been included for purposes of comparison. Serum tocopherol levels were deter-

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The third normal individual also ran two control and two vitamin E administration cycles (C1 and C2). The first control cycle showed a total of 13.9 mg. of pregnanediol excretion increasing to a total of 25 mg. when 50 mg. of vitamin E was taken daily. The second control cycle three years later showed a total of 18.8 mg. of pregnanediol, whereas the output during the vitamin E administration cycle was only 14.2 mg. The temperature

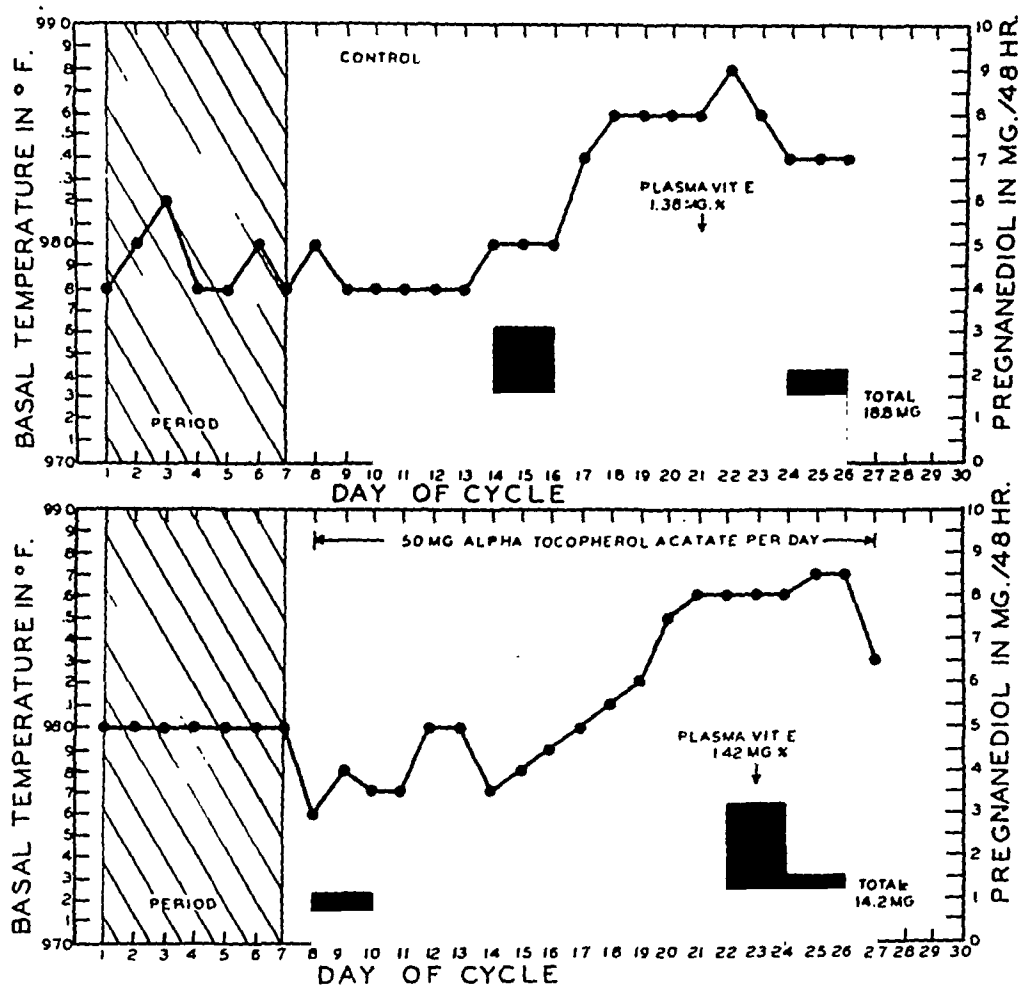


FIG. 2. Pregnanediol excretion blocked in milligrams for 48 hours is shown in relation to the day of the menstrual cycle and the basal body temperature. The first cycle illustrated is the control and the second is the menstrual cycle during which 50 milligrams of alpha-tocopherol was given each day.

charts obtained concomitantly with these pregnanediol excretion values are shown in Figure 2. The serum tocopherol level in this subject was 1.38 mg. per cent on November 26, 1947, at the height of the luteal phase in the control cycle. On December 7, immediately before vitamin E administration, the value was 1.07 mg. per cent and on December 23 during vitamin E administration the value was 1.42 mg. per cent, all within normal limits.

The fourth subject, D, who presented a sterility problem, had a total

mined before and during vitamin E administration on several subjects. Basal temperature charts on one individual were used as an aid in the analysis of luteal function.

METHODS

The pregnanediol excretion has been determined by the modified Astwood-Jones (6) technique, using 48-hour specimens in every case. The method is one which was devised in this laboratory and has been used constantly during the past eight years. Recovery of amounts of pregnanediol of less than one milligram for a 48-hour excretion period are considered unreliable and have been regarded as zero in the present calculations. Recovery of one milligram amounts has been consistent and such values are therefore considered accurate. Every precaution is taken in technique to make the determinations quantitative.

The serum vitamin E levels were measured by a modification of the Quaife-Harris method (7).

EXPERIMENTAL

Cyclic pregnanediol excretion for 5 individuals before and after vitamin E administration is shown in Figure 1. In addition, two normal cycles on a sixth individual have been charted.

There are three control cycles and three cycles during vitamin E administration shown on the first normal individual (A—1, 2 and 3). There is an increase of the total excretion from 34.1 mg. for the first control month, to 58.0 mg. for the month during which 50 mg. a day of vitamin E was administered. The following control month showed a total pregnanediol excretion of 29.4 mg., whereas the next cyclic excretion increased to 41.6 mg. when vitamin E in the same amount was again administered. The third feeding experiment was done on this subject four years after the original experiment and this time the total pregnanediol excretion in the control cycle amounted to 39.1 mg. When 50 mg. a day of vitamin E was administered, the excretion of pregnanediol, instead of rising, fell to 24.6 mg. The serum tocopherol before administration was 1.08 mg. per cent and following administration, 1.24 mg. per cent, both values being well within normal limits for the method used (8).

The next normal subject, B, showed a total urinary pregnanediol excretion of 34.4 mg. during a control cycle and 33.5 mg. when 50 mg. of vitamin E was taken daily. The second feeding experiment on this subject showed a total pregnanediol excretion of 40.5 mg. for the normal cycle and 20.3 mg. for the vitamin E administration cycle. It may be noted that these values were obtained at the same time that those of A1 and A2 were determined.

effect upon the corpus luteum function of normal individuals when as much as 150 mg. a day of alpha-tocopherol is given throughout the menstrual cycle. From the present experiment it is not possible to say whether abnormal individuals or individuals with a vitamin E deficiency, as indicated by the serum levels, would respond differently to the administration of alpha-tocopherol or whether a longer period of medication would lead to different results.

SUMMARY

1. The urinary pregnanediol excretion during 20 menstrual cycles of 6 apparently normal individuals has been studied. Eleven were control cycles and 9 were during periods of vitamin E administration.

2. Alpha-tocopherol serum levels were obtained on 2 of the subjects before and during vitamin E administration. These levels were within the normal range.

3. There is apparently a wide variation in the normal amount of pregnanediol excreted during the menstrual cycles of the same individual and a wider normal variation of excretion from individual to individual. No regular variation in pregnanediol excretion was noted when alpha-tocopherol was administered in doses of 50 to 150 mg. per day for one month.

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pregnanediol excretion of 28.4 mg. in her control cycle with an increase to 34.8 mg. during the following month when 150 mg. a day of vitamin E was administered.

The fifth individual, E, was also a sterility problem. This patient had a total pregnanediol excretion of 35.6 mg. during her control month and the excretion remained at 35.9 mg. during the following month when she was given 50 mg. of vitamin E daily.

The final two cycles, F1 and F2 were normal control cycles (from the same individual). The first month, this individual excreted 44.6 mg. and the following month, a total of 31.3 mg. of pregnanediol.

DISCUSSION

Three cycles in the present study showed an increase in the amount of pregnanediol excreted during the vitamin E administration period over the amount of pregnanediol excreted during the control cycle (A1, A2, C1), two showed a decrease (A3, B2) and four showed no significant variation (B1, C2, D, E). The variations are further minimized when comparisons are made between the untreated cycles of the same individuals. Control cycles of subject A showed a total pregnanediol output of 31.4, 29.4 and 39.1 mg. Subject B showed somewhat less variation (34.4 mg. and 40.5 mg.) and subject C showed little variation (13.9 and 18.8 mg.). Subject F excreted 44.6 mg. in one cycle and 31.3 mg. in another, the difference of 13.3 mg. being the greatest variation in the present small series. In the light of these observations only one vitamin E feeding cycle (A1) could possibly be considered as increased over the normal variation, with an increase of 23.9 mg. Since a vitamin E feeding cycle in another individual, (A2) showed a decrease of 20.2 mg. rather than an increase, it would seem that even these relatively wide variations must be considered as perhaps due to some normal physiologic variation of corpus luteum function. This is in accord with the findings of Venning and Browne (9) and others.

The duration of the luteal phase also showed no constant variation during vitamin E administration. Four cycles were apparently increased (A3, C2, D, E), four were similar (A1, B1, B2, C1) and one (A2) was decreased in length over the normal control cycle. The variation in the duration of all the luteal phases was from a minimum of 12 days to a maximum of 18 days. The figure 18 was obtained as an estimation with the use of the temperature chart (Fig. 2). The duration of the menstrual cycles varied from a minimum of 23 days to a maximum of 32 days. The longest luteal phase, one of 18 days, occurred in a menstrual cycle lasting but 27 days, whereas the shortest of 12 occurred in a cycle of 24 days' duration.

Analysis of the material presented indicates that there is no constant

larly infrequent in eunuchs, but this question does not appear to have been studied critically.

A relative increase in androgen/estrogen ratio would seem to favor the appearance of asymptomatic hyperuricemia in an individual predisposed by heredity, and hence also to favor the later development of clinical gout. A qualitative alteration in androgen metabolism may also be important in the mechanism of gout. Wolfson and associates (4) reported very low outputs of urinary 17-ketosteroids in all of 11 gout patients studied. They were able to rule out all usual endocrine causes of this finding and to show that gout patients were able to transform exogenously introduced testosterone to ketosteroid at the usual rate. Because the hypogonadism ordinarily expected to accompany very low 17-ketosteroid outputs actually did not occur, they suggested that biologic androgen activity in gout was maintained by an abnormal androgen which, when metabolized, did not contribute importantly to urinary 17-ketosteroids. Since either men or women can inherit gout, they assumed the abnormal androgen to originate in the adrenal cortex. A second convergent series of recent studies has suggested participation of the pituitary-adrenocortical mechanism in attacks of acute gouty arthritis and in the regulation of urate metabolism (9-19).

Such observations lend interest to claims that the average plasma urate level of normal adult men is somewhat higher than that of normal adult women. Our data again confirm the existence of this sex differential and show that it occurs in the gouty as well as in normal adults. Results of renal clearance studies permit some preliminary insight into the mechanism by which this differential is maintained. The possible relationship of the normal sex differential to the mechanism of inherited hyperuricemia in gout will be discussed below.

METHODS AND SUBJECTS

Control subjects were normal young adults and ambulatory medical patients with minor ailments. The gout patients were research patients of the Department of Biochemistry and patients of the Arthritis Clinic. Unless otherwise stated, the urate values given are colorimetric total urate values rather than true urate values, as in our other clinical publications.

In carrying out studies of urate metabolism or excretion, it is important to avoid administering foreign substances whenever possible, since urate clearance is notoriously labile to pharmacologic influences. For this reason, glomerular filtration rates were estimated as $(1.75) \times (\text{urea clearance})$ with urine flows maintained constant or decreasing, and between absolute

been unable to influence gout with female sex hormones. Mara $\tilde{\text{n}}$ on (7, 8) on the other hand, has suggested intermittent estrogen therapy in gout, but has published no data on the results of such therapy.

THE TRANSPORT AND EXCRETION OF URIC ACID IN MAN. V. A SEX DIFFERENCE IN URATE METABOLISM

WITH A NOTE ON CLINICAL AND LABORATORY FINDINGS
IN GOUTY WOMEN*

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RECENT studies have tended to confirm the long suspected importance of the sex endocrines in gout (1, 2, 3, 4). Smyth, Cotterman and Freyberg (1) found that male relatives of gout patients who inherit asymptomatic hyperuricemia do not usually develop abnormally elevated plasma urate levels until after puberty. Stecher, Hersh, and Solomon (2) demonstrated that female carriers of this gene generally do not develop abnormally elevated plasma urate levels until the menopause, or the period of failing ovarian function which precedes the menopause. Because some years of hyperuricemia generally precede the onset of clinical symptoms, clinical gout follows a similar pattern, but at an interval of some years. Clinical gout in men seldom appears before puberty. In women symptoms ordinarily first occur after the menopause. Exceptionally, women do develop gout well before the menopause, possibly because of special hereditary factors (see addendum regarding gout in women). Hill (5) pointed out that in women with premenopausal gout, attacks of acute gouty arthritis tended to occur at the menses, the low point of estrogen secretion in the menstrual cycle.¹ Since the time of Hippocrates, gout has been reputed to be particu-

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¹ Attempts to treat gout with estrogens have apparently met with only indifferent success. We have seen no striking clinical change in two women with tophaceous gout and in one man with tophaceous gout who received rather large doses of stilbestrol. However, in a patient with pseudohermaphroditism and adrenogenital syndrome (Table 4), very large doses of stilbestrol appeared to cause some fall in plasma urate. Coste (6) has also

TABLE 2. RATIO OF AVERAGE FEMALE TO MALE PLASMA URATE CONCENTRATIONS IN REPORTED STUDIES ON NORMAL ADULTS (No distinction has been made between plasma and serum, both being included under plasma analyses. The term "Folin-Benedict" refers to color development technique which involves a phosphotungstate or arsenophosphotungstate color development with cyanide intensification. In all cases, a "direct" technique was used without previous isolation of urate as the silver salt. The method of Leone (23, 24) involves spectrophotometric determination of urate without previous deproteinization; all other techniques involve deproteinization.)

Authors	Material analyzed	Color development	Uricase	Normal men		Normal women		Female/Male Ratio
				No.	Plasma urate, mg. %	No.	Plasma urate, mg. %	
Present report								
Jacobson (20)	Plasma	Folin-Benedict	No	22	5.27	20	4.05	0.77
Mull (21)	Plasma	Folin-Benedict	No	63	4.40	37	4.00	0.91
Mull (21)	Plasma	Folin-Benedict	No	51	4.30	22*	3.81	0.89
Bulger and Johns (22)	Plasma	Folin-Benedict	Yes	51	3.23	22*	2.30	0.71
Leone (23, 24)	Plasma	Ferricyanide	Yes	62	4.40	41	3.40	0.77
Stecher <i>et al.</i> (3)	Plasma	Folin-Benedict	Yes	59	5.81	49	5.17	0.89
Brøchner-Mortensen (25)	Plasma	Folin-Benedict	No	396	3.95	294	3.63	0.92
Berglund and Frisk (26)	Plasma	Ferricyanide†	No	25	7.62	25	6.35	0.83
Brown (27)	Blood	Folin-Benedict	No	43	3.20	89	2.70	0.84
Median Female/Male ratio in all series summarized above: 0.84	Blood	Folin-Benedict	No	12	2.84	17	2.39	0.84

* In 37 normal pregnant women in the last trimester of pregnancy, the plasma total urate (no uricase) value averaged 3.59 mg. % and the true urate values (uricase), 2.24 mg. %. These values are 94% and 97% respectively of the values for normal nonpregnant women (21).

† Brøchner-Mortensen's technique is generally believed to give values which are somewhat too high.

urine flow limits of 12.0 and 2.0 ml. per minute. When this precaution is observed, the glomerular filtration rates estimated by this indirect procedure have been found to check well with inulin clearances or with endogenous creatinine clearances.

RESULTS

Table 1 compares the distribution of individual plasma urate values and the average plasma urate levels of the two sexes in four groups of

TABLE 1. DATA INDICATING A SEX DIFFERENTIAL IN PLASMA URATE CONCENTRATION BETWEEN MALE AND FEMALE NORMAL ADULTS AND BETWEEN MALE AND FEMALE GOUT PATIENTS

(Values for gout patients were determined while patients were on self-selection diets, without salicylate or cinchophen medication. Because more male than female patients were excluded from the table owing to uricosuric medication, the patients listed below are not a complete cross section of our gout series, so far as sex distribution is concerned.

Data on normal children are taken from a study conducted in cooperation with Dr. David Krevsky on urate metabolism in children. Note the absence of a sex differential in children.)

Range of plasma urate	Number of subjects							
	Normal children		Normal adults		Prethopaceous gout patients		Tophaceous gout patients	
mg. per 100 ml.	Male	Female	Male	Female	Male	Female	Male	Female
0.6 through 1.5	—	1	—	—	—	—	—	—
1.6 through 2.5	4	5	—	—	—	—	—	—
2.6 through 3.5	10	11	—	5	—	—	—	—
3.6 through 4.5	8	9	2	11	—	—	—	—
4.6 through 5.5	1	1	14	4	—	1	—	—
5.6 through 6.5	1	—	6	—	—	1	—	—
6.6 through 7.5	—	—	—	—	4	3	—	1
7.6 through 8.5	—	—	—	—	12	2	—	—
8.6 through 9.5	—	—	—	—	5	—	1	—
9.6 through 10.5	—	—	—	—	2	—	3	1
10.6 through 11.5	—	—	—	—	1	—	4	—
11.6 through 12.5	—	—	—	—	—	—	2	—
12.6 through 13.5	—	—	—	—	—	—	—	—
13.6 through 14.5	—	—	—	—	—	—	—	1
<i>Average plasma urate</i>								
mg. per 100 ml.	3.39	3.21	5.27	4.05	8.10	6.70	11.05*	10.20*
<i>Sex differential in plasma urate, mg. per 100 ml.</i>								
	0.18		1.20		1.60		0.85	

* Median values.

The ratio of urate clearance to glomerular filtration rate is somewhat higher in the women than in the men of either group. Analysis of the mechanism is better carried out by reference to the control group in order to avoid alterations secondary to renal impairment. In the control group, the urate clearance is somewhat greater in the women than in the men, whereas glomerular filtration rate is somewhat greater in the men. Both differences operate toward making the ratio of urate clearance to glomerular filtration rate lower in men.

TABLE 3. AVERAGE URATE CLEARANCE DATA IN CONTROL AND GOUTY SUBJECTS
(All data are given per 1.73 sq.M. of calculated surface area)

Subjects	Plasma urate, mg. %	Urine urate, mg./min.	Urate clearance, cc./min.	Glomerular filtration rate, cc./min.	^c Urate G.F.R.
<i>Control subjects</i>					
9 males	5.38	0.558	10.38	119	0.087
11 females	4.14	0.524	12.67	116	0.109
<i>Gout patients</i>					
14 males	7.88	0.453	5.74	64	0.089
5 females	7.58	0.435	5.74	57	0.101

The decrease in clearance ratio appears almost entirely to account for the sex differential in plasma urate level. Differences in the actual amount of urate excreted per minute per unit surface area are slight and do not approach the magnitude of the difference in clearance ratio. Brøchner-Mortensen's data (25) show the same general tendency, with a ratio of urate to exogenous creatinine clearance of .063 for normal men and .072 for normal women.

Table 3 also suggests the importance of comparing gout patients with control subjects of the same sex when studying urate excretion. It is apparent that comparison of a gout group composed chiefly of male patients with a control group composed chiefly of women would lead to the erroneous conclusion that the ratio of urate clearance to glomerular filtration rate is significantly reduced in gout. Ultimately, this may prove to be the case, but it is not demonstrated by the data of Table 3.

DISCUSSION

The average plasma urate concentration of normal adult women is about 84 per cent of that of normal adult men. This sex differential appears

subjects: normal children, normal adults, patients with pretophaceous gout, and patients with tophaceous gout. In each group, except children, men have higher average urate levels than women, although there is considerable overlap in the distributions.

Tophi are usually a late development in gout and perhaps represent a complication rather than an integral part of the disease. Plasma urate levels in tophaceous gout tend to be considerably higher than in pretophaceous gout, but this may be due to the greater degree of renal impairment in patients with tophi. Fifteen of our patients with pretophaceous gout have been found to have an average glomerular filtration rate of 76 cc./min./1.73 sq.M., whereas 5 patients with tophaceous gout had an average of only 44 cc./min./1.73 sq.M.

Because of this somewhat equivocal status of the subcutaneous tophus, a better measure of the constitutional factor which produces gouty hyperuricemia is the magnitude of the difference in plasma urate concentration between the average patient with pretophaceous gout and the average normal adult of the same sex. In our series, this difference, calculated from the data of Table 1, is 2.83 mg. per cent in men and 2.65 mg. per cent in women. In other words, the genetic factor appears to produce an approximately equal elevation of plasma urate level in both sexes, when the norm is taken as the average adult plasma urate concentration of normal individuals of the same sex.

Table 2 summarizes the results of a number of studies in which urate levels of normal men and women have been compared. All recent studies show the existence of the sex differential. The average ratio of female/male plasma urate levels appears to be about 0.84. Two factors appear to explain the rarity of clinical hyperuricemia in casual groups of adult females. The more important factor is the lack of recognition of the normal sex differential; but an additional cause must be the failure to recognize that inherited hyperuricemia in women is not usually completely manifested until after the menopause (3). There is, in addition, some suggestion that urate levels are higher in postmenopausal nongouty women than in premenopausal women (3).

The only large series of comparative data on gouty men and women is that of Hill (5). In 59 men with typical articular gout, Hill found that 44 per cent had blood urate concentrations above 5.5 mg. per cent, whereas only 25 per cent of 16 gouty women had similarly elevated levels. In a number of published series which have included a few gouty women, the average blood urate concentrations of the women have been less than those of the men. This is true of the reports of Brøchner-Mortensen (25), Talbott (28), Stecher (29), Herrick and Tyson (30) and Gibson and Kersley (31).

Table 3 summarizes clearance studies on gouty and control subjects.

num effect until the same period. A final differential is that which obtains between the average plasma urate of patients with pretophaceous and tophaceous gout. This averages about 3.3 mg. per cent. So far as we are aware, no one has ever studied the question of whether the severity of gout is inherited or whether the tendency to tophaceous gout is inherited, but the earlier onset of tophaceous gout in women and the more frequent family history obtained from such patients is suggestive. Most interesting of all are the occasional reports of a family history of gout in women (see the appended note on gout in women).

The amount of urate excreted per minute is about equal in the control men and women and in the gouty men and women. The higher plasma urate of normal men appears to be a function of the lower ratio of urate clearance to glomerular filtration rate in this sex. In turn, the smaller clearance ratio of men seems to arise from lower urate clearances and higher glomerular filtration rates in normal men. The sex differential in plasma urate, superficially at least, appears to reflect differences in the urate excretory mechanism³ between the two sexes, rather than differences in the rate of purine turnover.

Relationship of the normal sex differential to inherited hyperuricemia. The tendency to develop hyperuricemia is inherited,⁴ but the age at which this latent tendency is translated into an elevated plasma urate level seems to depend in part upon endocrine status. In either sex, actual hyperuricemia ordinarily develops at a period of life characterized by a relative increase in androgen unantagonized by estrogen. In men, both the normal sex differential and inherited hyperuricemia produce their characteristic elevations in plasma urate level at, or shortly after, puberty. At first, this might suggest that inherited gouty hyperuricemia might really be only an exaggeration of the normal sex differential, a form of inherited hyperandrogenism.

A major obstacle to interpreting inherited hyperuricemia as a type of inherited hyperandrogenism is the complete lack of signs of such a condition in the gouty. Such a disturbance might perhaps be difficult to dis-

³ The problem of whether the difference in excretory mechanism responsible for the sex differential is ultimately to be referred to a difference in the transport mechanism for plasma urate, or to variations in a supposed tubular reabsorption of urate, will be discussed in detail elsewhere.

⁴ Most investigators now concede that the majority of all cases of gout are due to inherited factors. There remains some question as to whether severe renal impairment or increased nucleoprotein turnover (as in leukemia, lead poisoning and hemolytic anemia) can result in gout unless the inherited predisposition is also present. Since cases which raise the possibility of such "secondary" gout are distinctly unusual, they are omitted from present consideration.

to arise at puberty, since it is not found in normal children,² and presumably is related to the increased rate of secretion of normal androgen which occurs at puberty. There is some rise in the plasma urate of normal females at puberty, which is to be expected since adrenal androgen production in females reaches adult levels at this time but the rise is considerably greater in normal males, presumably because of their quantitatively greater secretion of normal androgen. A similar sex differential occurs in patients with gout. The average plasma urate concentration of gouty men is higher than that of gouty women, whether gout patients are considered as a group or subdivided according to the presence or absence of subcutaneous tophi.

It is possible to give a rough quantitative analysis of factors tending to cause elevated plasma urate concentrations. One may consider the average plasma urate of normal children, 3.3 mg. per cent, to be a basal level. At puberty, normal women receive an increment averaging 0.8 mg. per cent in plasma urate, presumably reflecting adrenal androgen secretion. Men, who have considerably more androgen arising from both testes and adrenals, receive a larger increment, averaging 2.0 mg. per cent. The problem of the quantitative increase occurring in the normal woman at the menopause is under study by Smyth and his coworkers, but as yet detailed information is not available.

The increment representing the effect of normal androgen secretion (2.0 mg. per cent) is added to all urate levels in the male at puberty, whether or not the individual has inherited the gene for hyperuricemia. However, inheritance of this gene results in the addition of a further increment, averaging 2.7 mg. per cent in either sex, to the normal adult levels for that sex. It is interesting that although the magnitude of the increment controlled by the gene for hyperuricemia ultimately appears to be equal in the two sexes, the period at which the maximum urate levels are reached differs in the two sexes. One might suggest that, just as maximum urate levels are not reached in the normal woman until normal ovarian function terminates, so also the gene for hyperuricemia may not produce its maxi-

² Several other interesting and pertinent observations were noted in the more detailed analysis of urate metabolism in children (32). The appearance of the sex differential in plasma urate is distinctly a puberal phenomenon, since the urate levels for both sexes show no differential in the age 0-6 group or in the age 7-13 group. The average plasma urate for all individuals is only very slightly higher in the age 7-13 than in the age 0-6 group. Values for the ratio of urine urate to preformed creatinine, urine urate to total creatinine, and urate clearance to creatinine clearance were very considerably higher in children than in adults, and preliminary findings suggest that this entire pattern of differences may be diminished temporarily by the administration of potent androgens. These results will be reported in detail in future communications.

tory evidence of hyperandrogenism in gouty men, the mutual independence of the sex differential and inherited hyperuricemia, and the failure of gouty hyperuricemia to exhibit an invariable dependence upon endocrine status all oppose interpretation of inherited gouty hyperuricemia as merely an exaggeration of the normal sex differential due to inherited hyperandrogenism. Since the facts nevertheless point toward some relationship between the factors which control the appearance of the sex differential and of inherited hyperuricemia, this relationship must be somewhat indirect. As data now available actually do suggest the existence of a qualitative abnormality of androgen secretion in the gouty (4), a plausible hypothesis might be that the gouty possess a second hormone with a rate of secretion parallel to that of normal androgen under most circumstances, opposed by estrogen like other androgens, and responsible for the manifest expression of inherited hyperuricemia.

The working hypothesis. Present evidence indicates that the greater frequency of abnormally elevated plasma urate levels in male relatives of the gouty than in female relatives does not mean that more men than women inherit the gene for hyperuricemia. Rather the difference in the sex incidence of abnormal urate levels depends upon two nonhereditary factors: a) the fact that male carriers develop their maximum urate levels at an earlier age than female carriers, and b) the existence of the normal sex differential in plasma urate concentration (1, 2, 3). Since a prolonged and severe hyperuricemia seems to favor the appearance of clinical gout, the sex incidence of the disease may be assumed to depend upon the male tendency to earlier and greater hyperuricemia.⁵

The simultaneous appearance of the sex differential and of inherited hyperuricemia at puberty in the male, and of inherited hyperuricemia at the menopause in women, has suggested that gouty hyperuricemia might be a sort of exaggerated sex differential due to inherited hyperandrogenism. We have already reviewed the objections which appear to make this uncomplicated view untenable and which favor a more indirect type of relationship. The finding of low urinary 17-ketosteroids in the gouty, in the presence of clinically normal androgenic status and unimpaired ability to convert administered testosterone to ketosteroids, is believed to indicate that biologic androgen activity is maintained in the gouty by an abnormal androgen which, when metabolized, is not importantly excreted as 17-ketosteroid.

The present data are consistent with the assumption that the abnormal

⁵ The observation that the occurrence of clinical gout and the duration and magnitude of antecedent hyperuricemia are positively correlated should not be taken to mean that the elevated plasma urate level is necessarily the direct cause of the symptoms of clinical gout.

cover in normal adult males, but should certainly be detectable as virilism in gouty females. However, data in the note appended indicate that virilism does not occur in gouty females. It is interesting that this lack of virilism agrees with observations of Draper, Dupertuis and Caughey (33) on the constitutional status of gouty females. They report "strong emphasis on gynec features, with minimal emphasis on the andric." A laboratory finding to be expected if gouty hyperuricemia were due to inherited hyperandrogenism would be increased 17-ketosteroid excretion in the urine of gouty patients. The actual data show very low urinary ketosteroids to be characteristic of the gouty (4). For reasons already mentioned, these low ketosteroid outputs are believed to be referable to secretion of an abnormal androgen which is not metabolized to ketosteroids.

An important further objection to considering inherited hyperuricemia as an exaggerated normal sex differential is the occurrence of a sex differential in the gouty as well as in normals. This suggests that the mechanisms controlling the sex differential and inherited hyperuricemia cannot be entirely coincident but must have some degree of independence from each other. Moreover, inherited hyperuricemia seems to consist of an elevation of plasma urate over a basic level set by the individual's sex; but the magnitude of this elevation is about equal in the two sexes and not particularly greater in gouty males, as would be expected if it depended upon the rate of androgen secretion.

Finally, it is important to note that the appearance of gouty hyperuricemia, while ordinarily conditioned by factors which seem to be dependent on androgen/estrogen ratios, may exceptionally be quite independent of such control. The data on gouty women which follow illustrate the fact that functioning ovaries may provide only relative protection against gout, since tophaceous gout in women seems generally to begin before the menopause. In the 3 cases of tophaceous gout in women for whom we know the actual date of the menopause, the onset of clinical gout preceded the menopause by 7, 20, and 21 years respectively. In 3 other women with tophaceous gout, the disease began at ages 12, 16, and 26 respectively. If one may credit the older clinical literature, some modification must also be made in the Hippocratic aphorism that gout is absent in eunuchs, since Cullen (34) found it necessary to add that gout could occur in eunuchs, if they "happen to be of a robust habit, to lead an indolent life, and to live very full." There are also exceptions to the relative freedom from gout enjoyed by children. In most cases, gout in children appears to be a complication of increased nucleoprotein turnover consequent upon leukemia or hemolytic anemia, but in some authentic cases no such factor can be found (35, 36, 37).

The lack of virilism in gouty women, the absence of clinical or labora-

TABLE 4. PLASMA URATE CONCENTRATION IN CERTAIN ENDOCRINE
AND OTHER SYNDROMES

(All patients in the table below are genetic females. The only endocrine disturbance in which altered urate metabolism has regularly been reported is active acromegaly (39-47). Most investigators have reported low urate levels and high urate outputs in active cases. Thannhauser (41) was able to reverse these changes by pituitary irradiation in one acromegalic patient. Irradiation also caused a marked drop in urine urate/urine creatinine. No change was observed in a control subject similarly irradiated.)

Num- ber of patients	Num- ber of samples	Plasma urate, mg. %	Plasma urea, mg. %	Urate —— urea	Therapy and remarks
Normal adult women					
20	20	4.05	27.9	0.15	All premenopausal subjects
Acromegaly					
1	1	2.70	30.0	0.09	Early active acromegaly
1	1	4.60	43.0	0.11	Late "burned-out" acromegaly; meta- hypophyseal diabetes mellitus
Cushing's syndrome*					
2	4	3.89	36.2	0.11	Preoperative.
2	3	5.93	8.5	0.70	Postoperative. Successful removal of adrenal adenoma with clinical cure in both cases.
Nephrotic syndrome (26 pound child)*					
1	3	5.70	32.0	0.18	Before ACTH treatment
1	1	2.00	34.6	0.06	ACTH, 3×5 mg. for 4 days
1	2	4.10	24.4	0.17	First week after ACTH
1	2	3.30	18.4	0.18	Second week after ACTH. Beginning of one month clinical remission
Pseudohermaphroditism with adrenogenital syndrome					
1	1	8.20	27.0	0.30	No therapy
—	—	—	—	—	Abdominal exploration. Unilateral oophorectomy and biopsy of second ovary. Nests of lutein cells with ac- tive mitotic figures in both ovaries
1	1	7.30	33.0	0.22	Stilbestrol, 5 mg. daily
1	1	7.70	20.0	0.38	Stilbestrol, 50 mg. daily
1	1	7.10	27.0	0.26	Stilbestrol, 65 mg. daily
1	1	6.10	27.0	0.23	Stilbestrol, 100 mg. daily
1	2	5.90	33.0	0.18	Removal of left adrenal adenoma; not cured.
Gout in women					
14	21	7.45	39.6	0.19	See Table 5 for further details

* See p. 761.

androgen of the gouty is one which has the particular property of producing an excessive elevation in plasma urate concentration, that it ordinarily, but not always, begins to be secreted at the same period of life when normal androgen secretion begins, and also that it is functionally opposed by estrogen. The "gouty androgen" is presumed to be of adrenocortical origin, since gout may be inherited by either men or women. Albright (38), in particular, has emphasized the fact that, even in normal individuals, the onset of adrenal androgen secretion ("adrenarche") and its cessation ("adrenopause") may not exactly coincide with the onset of function or with involutional changes in the gonad. Similar timing discrepancies in adrenal-gonad relationship in the gouty may underlie the rare cases of constitutional gout in children and of premenopausal gout in women. The suggested adrenocortical origin of the "gouty androgen" makes gout in eunuchs understandable. One may speculate that some somatic action of the "gouty androgen" is reflected in the characteristic short, stout physique of the gouty woman, and in Cullen's gouty eunuchs "of a robust habit."

Since genes appear to control individual enzymes rather than individual tissues, it seems probable that an hypothesis which localizes the inherited defect in gout in disturbed adrenocortical function must at best be incomplete. Such an hypothesis has certain advantages notwithstanding; of these not the least is its consistency with other data which suggest altered adrenocortical function to be of central importance in the mechanism of the acute gouty attack. Attacks of acute gouty arthritis may be precipitated in a majority of interval gout patients by administering several doses of adrenocorticotrophic hormone (ACTH) and then withdrawing the hormone. The attack ordinarily begins from forty-eight to ninety-six hours after the hormone is withdrawn. It generally begins at that point during the temporary adrenocortical hypofunction following ACTH withdrawal when the normal subject shows evidence of abrupt "rebound" and temporary endogenous ACTH overproduction; but such evidence is lacking in the gouty, who show no rebound. The spontaneous attack of acute gouty arthritis or that produced by adrenocorticotropin-withdrawal may rapidly be terminated by the administration of adrenocorticotropin (9, 10, 19), but may return unless colchicine is also given (19). Metabolic and hematologic changes suggestive of temporary relative 11-oxysteroid deficiency are found during the prodromal periods of acute gouty arthritis of spontaneous origin, and the absence of metabolic findings of compensatory adrenocortical hyperfunction during prolonged attacks of acute gouty arthritis which resist treatment (9, 19) suggests that the gout patient may respond inadequately to relative 11-oxysteroid lack. Although these findings appear superficially to imply that a failure of tropic stimulation of the adrenal by the pituitary may be involved in the mechanism of acute gouty arthritis,

TABLE 5. CHARACTERISTICS OF GOUTY WOMEN

Reference	J.D. (47)	M.J. (47)	MCH. (47)	ANN. (47)	O.M. (46)	R.F. (46)	L.E. (47)	M.H. (47)	J.C. (47)	R.C. (47)	M.D. (48)	R.W. (49)	K.H. (28)	A.N. (28)	J.P. (30)	F.R. (30)	J.H. (50)	B.M. (35)	Average
Tophi	No	Yes	No	No	No	Yes	No	Yes	No	No	No	No	Yes	No	No	No	Yes	Yes	33%
Family history of gout	No	Yes	No	No	No	No	No	No	No	No	No	Yes	Yes	—	—	—	Yes	Yes	31%
Height, inches	62.0	59.0	64.5	64.0	60.0	—	61.0	64.5	66.0	64.0	61.5	—	—	—	—	—	62.4	62.4	62.7
Weight, pounds	155	133	200	210	170	—	162	173	170	175	140	185	151	—	—	150	180	168	163
Age at onset of gout	55	38	50	51	57	—	47	26	49	55	59	68	14	61	55	69	55	26	49
Age at menopause	50	45	51	50	42	—	—	57	44	46	55	—	34*	—	—	—	—	—	47
History of toxemia of pregnancy	—	—	No	No	No	—	—	No	Yes†	No	—	—	—	—	—	—	—	—	16%
Para	—	0	3	2	3	—	6	3	2	2	—	—	—	—	—	—	—	—	2.6
Gravida	—	3	5	2	3	—	6	3	2	2	—	—	—	—	—	—	—	—	3.3
Hypertension	No	No	No	Yes	No	—	—	Yes	Yes	Yes	—	—	—	—	—	—	—	—	58%
Serum urate, mg. %	5.9	13.7	8.4	8.0	6.7	7.4	6.7	10.2	5.0	6.8	7.0	7.0	Yes	No	—	—	Yes	Yes	39.1
Serum urea, † mg. %	48	30	33	38	37	25	34	79	35	33	—	—	41	36	—	—	10.6	9.7	8.1
Hemoglobin, Gm.	10.8	12.1	12.1	11.9	11.8	—	13.3	13.7	14.1	13.1	—	—	—	—	—	—	14.7	15.3	39.6
Erythrocytes, millions per cumm.	3.97	3.72	4.04	4.46	4.45	—	4.06	4.40	—	4.31	—	—	4.15	—	—	—	4.70	5.14	4.31
Hematocrit, vols. %	—	34	43	41	—	—	40	45	—	38	—	—	37	—	—	—	—	—	39.7
Color index	0.87	1.04	0.96	0.85	0.85	—	1.05	1.00	—	0.97	—	—	—	—	—	—	1.00	0.94	0.97
Mean corpuscular volume	—	0.91	1.07	0.92	—	—	0.98	1.02	—	0.88	—	—	0.89	—	—	—	—	—	0.92
Total leukocytes, §	9.1	4.9	8.4	8.9	8.0	—	10.6	5.0	—	—	—	—	6.3	—	—	—	8.0	8.8	7740
Polymorphonuclear cells	67	61	36	65	49	—	—	46	—	—	—	—	—	—	—	—	84	58	54.5%
Eosinophils	3	2	4	1	0	—	—	1	—	—	—	—	—	—	—	—	0	0	1.6%
Basophils	0	1	0	0	0	—	—	1	—	—	—	—	—	—	—	—	0	0	0.3%
Lymphocytes	25	28	56	25	46	—	—	48	—	—	—	—	—	—	—	—	16	38	38.0%
Monocytes	5	8	4	9	5	—	—	4	—	—	—	—	—	—	—	—	0	4	5.6%
Glomerular filtration rate, cc. per min. per 1.73 sq.M.	—	54	—	—	73	—	—	33	85	59	—	—	12	—	—	—	—	—	52.7
Serum total cholesterol, mg. %	323	197	196	199	—	—	—	—	—	—	—	—	—	—	—	—	—	—	229
Cholesterol esters	67%	70%	73%	69%	—	—	—	—	—	—	—	—	—	—	—	—	—	—	70%
Thymol turbidity, units	3.0	2.1	1.0	1.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.9
Thymol flocculation	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Normal
Cephalin flocculation	0	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Normal
Gold flocculation	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Normal
Serum bilirubin mg. %	0.2	0.4	—	0.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4
Serum total protein Gm. %	8.1	7.9	7.0	7.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7.5
Hove "albumin" Gm. %	4.7	5.5	4.5	4.8	—	—	—	4.4	—	—	—	—	—	—	—	—	—	—	4.8
Hove "globulin" Gm. %	3.4	2.4	1.8	2.2	—	—	—	3.0	—	—	—	—	—	—	—	—	—	—	2.7
True albumin % T.P.	44%	49%	—	—	—	—	—	45%	—	—	—	—	—	—	—	—	—	—	46%
Alpha globulin % T.P.	14%	21%	—	—	—	—	—	14%	—	—	—	—	—	—	—	—	—	—	16%
Beta globulin % T.P.	9%	14%	—	—	—	—	—	18%	—	—	—	—	—	—	—	—	—	—	14%
Gamma globulin % T.P.	33%	16%	—	—	—	—	—	23%	—	—	—	—	—	—	—	—	—	—	24%

* Surgical menopause
† Eclampsia during first pregnancy, pre-eclampsia during second.
‡ When NPN values were given, urea nitrogen was assumed to constitute 50% of the NPN.
§ The values on patient J.H. were obtained during acute gouty arthritis, and are omitted from the averages.

a sharp drop in serum cholesterol (averaging 63 mg. per cent) which may occur early in the attack without other evidence of increased 11-oxysteroid output suggests that the adrenal is manufacturing steroids other than 11-oxysteroids at an increased rate (14).

These clinical findings which indicate 11-oxysteroid production by the adrenal to be important in the mechanism of acute gouty arthritis are paralleled by evidence that these steroids regulate urate metabolism. Table 4 presents data which suggest that altered adrenal function may be important in clinical disturbances of the plasma urate level. Numerous recent physiologic studies have indicated 11-oxysteroids to increase both the rate of urate production and urate clearance (15-19), whereas, in children, androgens may be shown to have opposing effects (32).

Our present working hypothesis assumes that, in gout, biologic androgen activity is maintained by the secretion of an abnormal adrenocortical androgen which, when metabolized, is not importantly converted to urinary 17-ketosteroid. Like other androgens, this "gouty androgen" is estrogen-antagonized. Within the limits of the flexible adrenal-gonad relationship, this androgen is secreted at the times when normal androgen is secreted. The "gouty androgen" is not necessarily responsible for attacks of acute gouty arthritis, since a gouty adrenal which secretes an abnormal androgen is reasonably suspect of responding to pituitary adrenotropic stimulation in an unusual fashion. However, the onset of clinical gout appears in most cases to be correlated with the duration and severity of antecedent hyperuricemia (1, 2). Since the abnormal androgen appears to regulate the time of appearance of inherited hyperuricemia in those genetically predisposed, its secretion would appear basic to the susceptibility to clinical gout.

CONCLUSION

1. The average plasma urate of men is somewhat higher than that of women, both in the normal and gouty groups. The differences appear to depend on differences in the excretory mechanism for urate. These differences are believed to depend upon normal androgen since they are not present before puberty.

2. Carriers of genetic hyperuricemia, an inherited biochemical lesion of gout, have been found to have normal plasma urate levels until puberty, if male, and until the menopause, if female. This suggests that androgen is

Footnote to Table 4.

* Note the resemblance between the changes in plasma urate and urea caused by discontinuing ACTH therapy in the nephrotic child and those caused by successful removal of an adrenal adenoma in the patients with Cushing's syndrome.

The purified adrenocorticotropin used was furnished through the kind cooperation of Dr. John R. Mote, Medical Director, The Armour Laboratories, Armour and Company, Chicago.

may be inherited by either sex, this abnormal androgen presumably originates in the adrenal cortex.

5. *The working hypothesis.* Our preceding report suggested that, in gout, biologic androgen activity is maintained by an abnormal androgen of adrenocortical origin which, when metabolized, does not contribute importantly to urinary 17-ketosteroids. The present study suggests that an abnormal androgen of adrenocortical origin is responsible for the appearance of an abnormally elevated plasma urate level in those genetically predisposed. This abnormal androgen appears ordinarily to be secreted under the same conditions as normal androgen and to be estrogen-opposed.

Tentatively, we have assumed the two abnormal adrenocortical androgens of the preceding paragraph to be identical. Since the onset of clinical gout appears in most cases to be correlated with the duration and severity of antecedent hyperuricemia, and since the abnormal adrenocortical androgen appears to regulate the time of appearance of hyperuricemia in those genetically predisposed, its secretion would seem to be basic to the susceptibility to clinical gout. The attack of acute gouty arthritis, on the other hand, appears more closely related to a deficient response to temporary relative 11-oxysteroid lack. The possible role of the abnormal adrenocortical androgen of gout in the production of the characteristic acute gouty arthritis remains to be clarified.

Addendum

A note on the clinical and laboratory findings in women with gout.

The accompanying paper has indicated the importance of the presence or absence of virilism in gouty women when considering an endocrine hypothesis of gout. Since there appears to have been no summary of data published on a sizable group of gouty women, this added note is intended to serve that purpose.

Table 5 summarizes individual findings in 18 gouty women. Ten patients were from our series (47) and data on the other 8 were taken from recent publications (25, 28, 30, 48, 49, 50). Virilism was not reported by any investigator, and did not occur in our group. There does appear to be a characteristic physique, consisting of marked obesity and relatively short stature. In addition to the available height and weight values in Table 5, several other reports mention this body configuration without actually giving heights or weights.

Table 6 clearly shows the marked differences between tophaceous and pretophaceous gout in women. Tophaceous gout in women begins before the menopause. It is associated with considerably more impairment of renal function and with higher plasma urate levels than pretophaceous gout. A family history of gout was obtained in more than half of the

TABLE 6. CONTRASTS BETWEEN TOPHACEOUS AND PRETOPHACEOUS GOUT IN WOMEN.*

(This table includes the patients summarized in Table 5 and two additional women with tophaceous gout reported by Bernstein (35).)

	Tophaceous gout	Pretophaceous gout
Number of patients	8	12
Family history of gout	63%	20%
Average age at onset of gout	27	56
Onset before menopause	100%	14%
Average height, inches	62.0	63.3
Average weight, pounds	157	174
Ideal weight, 1912 tables	136	124
Average overweight, per cent of ideal weight*	15%	23%
Average serum urate, mg. %	9.6	6.9
Average serum urea; mg. %†	43.5	36.8
Glomerular filtration rate, cc./min./1.73 sq.M.	33	72
Hypertension	67%	42%
Diabetes mellitus	0	2
Hematopoietic disease	1‡	0

* Obesity may have been minimized in the tophaceous group by the greater severity of renal impairment.

† Values are for urea, as urea. In certain of the published cases reviewed, urea values were calculated from NPN values by assuming urea-N to form 50% of the NPN.

‡ Chronic monoblastic leukemia.

important in the conversion of latent genetic hyperuricemia into an actual elevation in plasma urate.

3. Since both appearance of the normal sex differential and of inherited hyperuricemia appear favored by androgen, we have considered the possibility that inherited hyperuricemia is merely an exaggeration of the normal sex differential resulting from inherited hyperandrogenism. This suggestion appears incorrect. Virilism does not occur in gouty women. No findings suggest hyperandrogenism in gouty men. There is, in fact, evidence to indicate that gout patients secrete less, rather than more, normal androgen than do normal adults.

4. The existence of a sex differential in the gouty as well as in normals suggests that separate androgenic mechanisms regulate the sex differential and the transformation of latent inherited hyperuricemia to the manifest hyperuricemia which precedes gout. Since hyperuricemia is of restricted occurrence, the latter would appear to be an abnormal androgen with a particular ability to cause an elevated plasma urate. Since hyperuricemia

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patients with tophaceous gout, but in less than a quarter of those with pretophaceous gout. This suggests that the earlier onset and greater severity of tophaceous gout in women is not due to casual factors, but is in some way genetically determined.

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THE CONSTANCY OF THE SERUM PRECIPITABLE OR PROTEIN-BOUND IODINE IN HEALTHY ADULTS

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IT has been demonstrated that the level of serum precipitable or protein-bound iodine varies with thyroid activity (1-6). As a result of these studies it is evident that in healthy subjects without disorders of this gland the organic iodine fraction in plasma is usually somewhere between 4 and 8 gamma per cent. It is not known, however, whether or not this serum precipitable or protein-bound iodine value changes from time to time within this range in any euthyroid individual. This possibility cannot be excluded in view of the knowledge that not only the level of thyroid activity but also extra-thyroidal factors may alter the value of the organic iodine fraction in serum (7, 8). The studies herein reported establish, however, the relative constancy of the serum precipitable iodine in healthy young adults, male and female, during a period of several weeks.

MATERIALS AND METHODS

Levels of serum precipitable or protein-bound iodine were measured at about weekly intervals in 4 women and 2 men, 21 to 32 years of age, during periods of one and a half to two months. In all of the female subjects the onset and duration of menses were recorded. In addition, 2 women kept a daily chart of the body temperature (oral) determined during a five-minute period before arising.

Venous blood was withdrawn in the morning, one to three hours following a light, or in the case of one of the male subjects (R.G.), a complete breakfast. The precipitable iodine concentration was measured by the method of Barker (9) with slight modifications (10). Recoveries of potassium iodide, diiodotyrosine, and thyroxine were 95 per cent and better in concentrations corresponding to the serum values obtained in this study.¹ In 2 studies, J.M. and J.G., the sera were analyzed shortly following the

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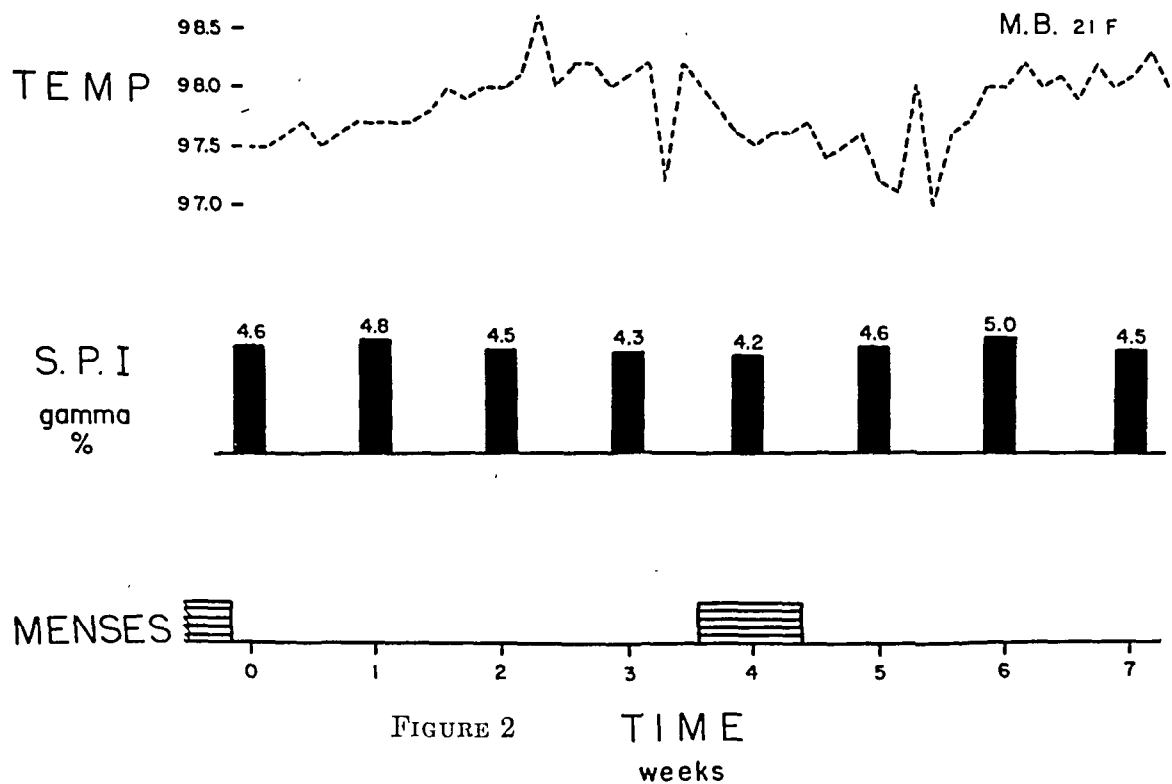


FIGURE 2

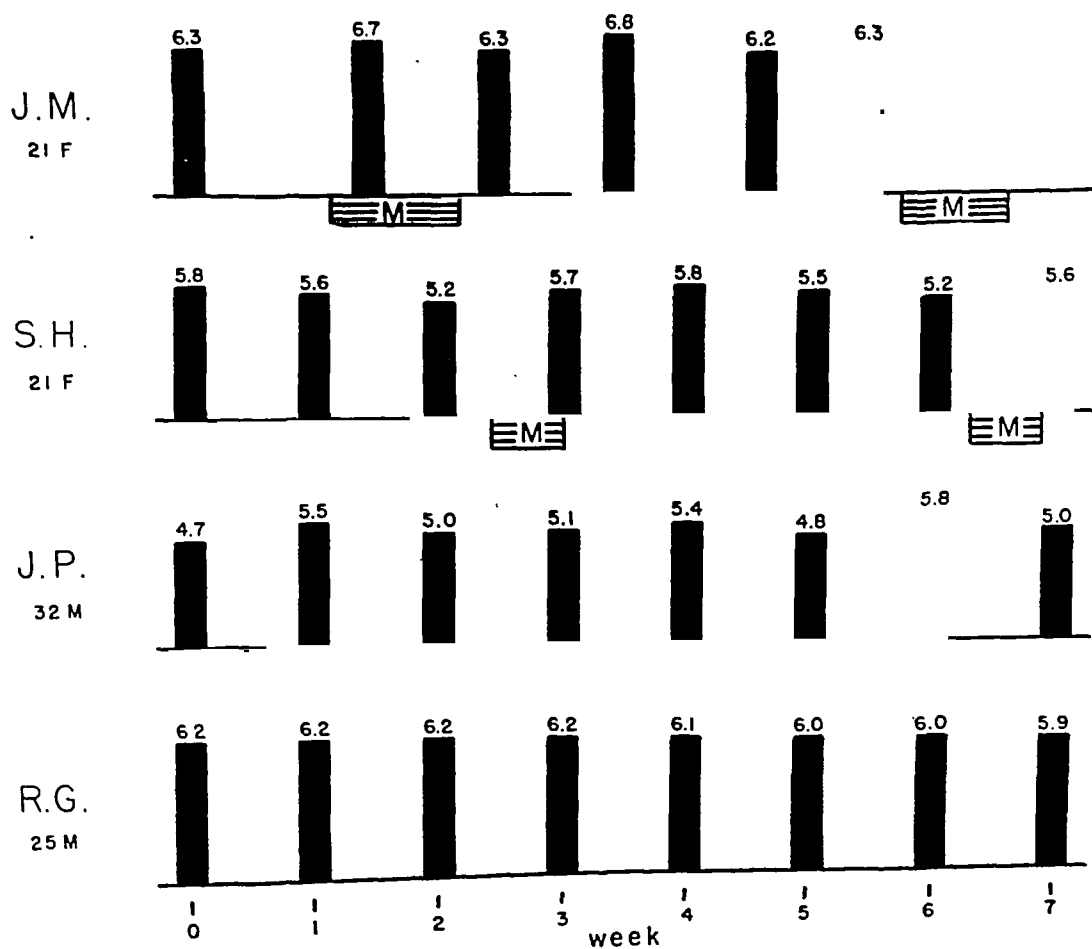


FIGURE 3

venipuncture. In the other 4 the sera were precipitated and washed in the usual manner, stored at -10°C . until the completion of the experiment, and then analyzed as a group. Results of analyses in duplicate or triplicate agreed, on the average, within less than 0.3 gamma per cent. The findings are presented in Figures 1 through 3.

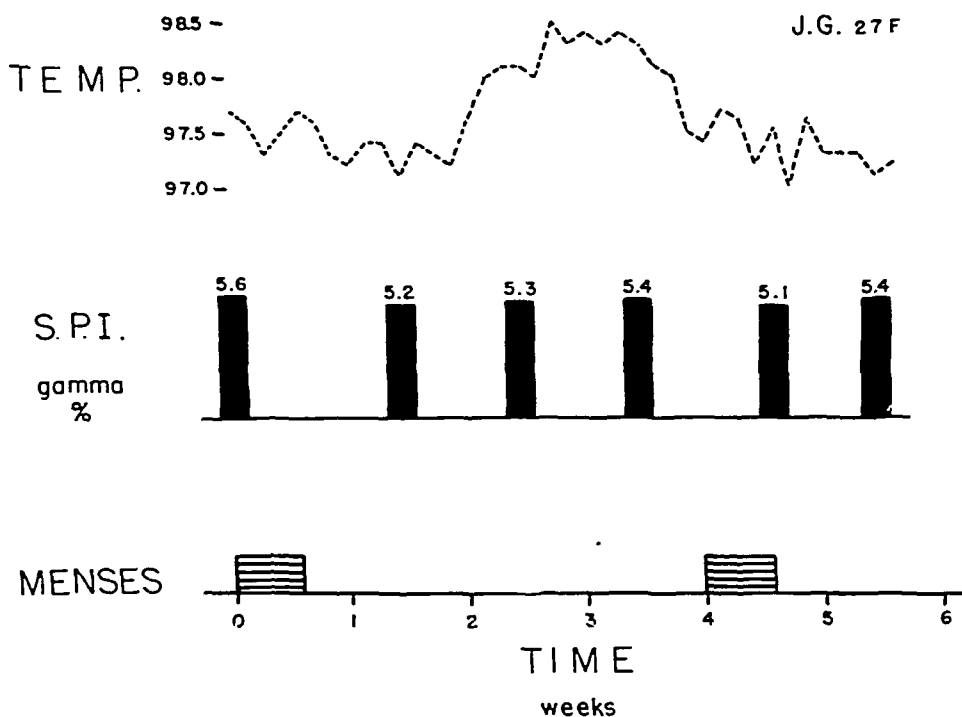


FIGURE 1

DISCUSSION

All of the individuals studied appear to have had euthyroid levels of serum precipitable iodine, falling within the 4 to 8 gamma per cent range described for subjects without thyroid disorder. The maximum variations in the serum precipitable iodine in any one of the 4 women were only 0.5 to 0.8 gamma per cent during the 6 to 8 analyses which were made. The smallest variation, 0.3 gamma per cent, was observed in R.G., one of the two male subjects (Fig. 3). This particular subject, it will be recalled, was the only one with a regular intake of a complete breakfast one to two hours prior to withdrawal of the blood sample. The largest change in the serum precipitable iodine, 1.1 gamma per cent, occurred in the second male subject (J.P., Fig. 3). It is clear from these findings that the serum precipitable or protein-bound iodine concentration varies but slightly from week to

showed the characteristic rise during the postovulatory phase of the cycle (16, 17). This terminated with a drop in the basal temperature just before or with the onset of menses. It seems reasonable to assign some, and perhaps all, of the sporadic dips or peaks in body temperature during the trends, to extrinsic factors unrelated to the menstrual cycle itself. Analyses of the precipitable or protein-bound iodine were run on serum obtained on one or more occasions within a few hours of the onset of menses, just after the onset, during the period itself, and shortly following its termination. Also, it is probable that the ovulatory phase was covered in a similar manner although conclusive evidence for this is, of course, lacking. Study of Figures 2 and 3 indicates, as might be expected, that it is not possible to detect during the menstrual cycle any definite elevation in serum precipitable or protein-bound iodine comparable to that observed early in pregnancy. However, if any trend is present, in subjects M.B., J.M. and S.H., it is toward a mid-point rise followed by a decrease during the postovulatory phase. It may be, therefore, that with pregnancy this slight rise is accentuated and maintained, and that in its absence the serum precipitable iodine declines to a lower level. The occurrence of equally great, if not greater fluctuations in the protein-bound iodine in one of the male subjects, J.P., might be viewed as evidence against such a theory. However, it by no means excludes the possibilities which have been presented, since thyroid function and levels of circulating protein-bound iodine do vary with factors other than pregnancy.

SUMMARY

1. The concentration of serum precipitable or protein-bound iodine appears to be relatively constant over a period of weeks in healthy young adults.

2. The hypothesis is cautiously advanced that the elevation in serum precipitable iodine which usually occurs in pregnancy may represent an accentuation and prolongation of a rise observed between menstrual periods.

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week. In evaluating the relative constancy of the serum precipitable iodine in these 6 subjects it should be kept in mind that they were all in good health. Their dietary habits remained fairly constant and presumably their levels of serum protein were unaltered. Neither did they receive inorganic iodide in any form other than that present in their usual diet, nor were they given any organic iodine compounds. Significant alterations in any of these factors are known to affect the levels of serum precipitable iodine (7, 8, 10).

The possibility cannot be excluded that some variations were occurring in these subjects in either the release of hormone from the thyroid gland or in its utilization or degradation. This is particularly true in view of the finding that in all but one of the subjects, the difference between the highest and lowest values exceeded the 0.3 gamma per cent analytical error. Inspection of the data presented graphically suggests that there may have been a cyclic variation in the level of serum precipitable iodine in some of the studies. To determine whether or not these variations are statistically significant the data were subjected to the following analysis: the "t" test was applied, using one or more sets of low and high mean values, and the dispersion of the individual values contributing to the mean.² The results of the "t" test together with the degrees of freedom, "n," were used to calculate a probability value, "p." In a total of 4 instances in 3 of the female subjects (M.B., J.M., and S.H.) "p" is less than 0.05, *i.e.* the observed differences could have occurred by chance only less than 5 times in a 100, and hence they may possibly be significant. No statistical significance can be assigned to the changes in the other female subject (Figure 1) since "p" is less than 0.30. The correlation of these slight but statistically significant changes in the serum precipitable iodine values with physiologic events is admittedly difficult and perhaps impossible. It is of interest, however, to speculate upon one possibility. Studies of the serum precipitable iodine in women indicate that the concentration of this fraction usually increases with pregnancy (11, 12, 13). This appears to be an early physiologic response, antedating the rise in the basal metabolic rate characteristic of the second and third trimesters (14, 15). Certain of the endocrine changes which characterize pregnancy, such as the decrease in follicular secretion and the rise in corpus luteum activity, also occur to some degree during the menstrual cycle. They may conceivably exert an effect on the serum precipitable iodine. Study of Figures 1, 2, and 3 reveals that in the course of periodic measurements of the serum precipitable iodine in the 4 female subjects probably all aspects of the menstrual cycle were covered. The 2 subjects who determined their daily basal temperature, J.G. and M.B.,

² Mr. Carrol S. Weil of the Mellon Institute kindly carried out the statistical analyses of the data.

A MASCULINIZING TUMOR OF THE OVARY IN A POSTMENOPAUSAL WOMAN

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THERE has been, and there still is, considerable confusion in the literature concerning certain masculinizing tumors of the ovary, especially the so-called adrenal rest tumor, or lipid cell virilizing tumor, or masculinovoblastoma, or hypernephroma of the ovary. The multiplicity of names, each including some characteristic of the tumor, bears testimony to the need for clarification.

In 1908 Bovin (1) reported the first case of hypernephroma or adrenal rest tumor of the ovary. Stadiem (2) reported 19 cases of hypernephroma of the ovary in 1937. In a critical review of these cases in 1939, Rottino and McGrath (3) could not classify any of them as virilizing lipid cell tumors of the ovary, and limited the total number reported in the literature to 7 cases. Kepler (4) in 1944 reviewed 6 additional cases and reported the fourteenth. Greene (5) in 1944 reported a case of adrenal rest tumor of the ovary, and Burket and Abell (6) later in the same year reported still another case. At the present time Curtis (7) declares that only 16 well authenticated and undisputed cases can be found in the literature. The case reported here can be added to the growing list. It is hoped that the information furnished by this report may add to the clinical and pathologic data so that eventually diagnostic criteria will catalogue this clinical entity more specifically.

CASE REPORT

R. R., XL 106160, a white female, aged 65, was admitted to the Indiana University Medical Center on January 17, 1947, complaining of weakness, and of "turning into a man." Four years prior to admission she began noticing hair on her face, body and extremities. She required shaving daily. At the same time her voice began to "crack" and became deeper than usual. Her skin became darker, and she developed pigmented hyperkeratotic spots on the face and body. The weakness complained of was in spells rather than continuously and on a few occasions resulted in a fall. It was relieved in part by taking something sweet. There had been no weight change, the patient always having been obese. She had a normal menopause at the age of 53.

In the past history, a diagnosis of diabetes was made at the age of 54, and again at 59, when she required up to 40 units of insulin daily. Later she discontinued both insulin and diet as she began to develop the weak spells of her present illness. For many years there had been a known hypertension as high as 240 systolic, with an average of 180 systolic.

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had numerous old surgical scars, but no striae. The viscera were not palpable and no masses were palpated in the renal areas. Pelvic examination revealed a clitoris $2\frac{1}{2}$ centimeters in length and 1 centimeter in diameter (Fig. 2B). There was a nulliparous vagina and a small soft cervix. The uterus showed multiple uterine fibroids on bimanual examination. Adnexal areas could not be adequately examined because of the obesity and the fibroids. The extremities were normal.

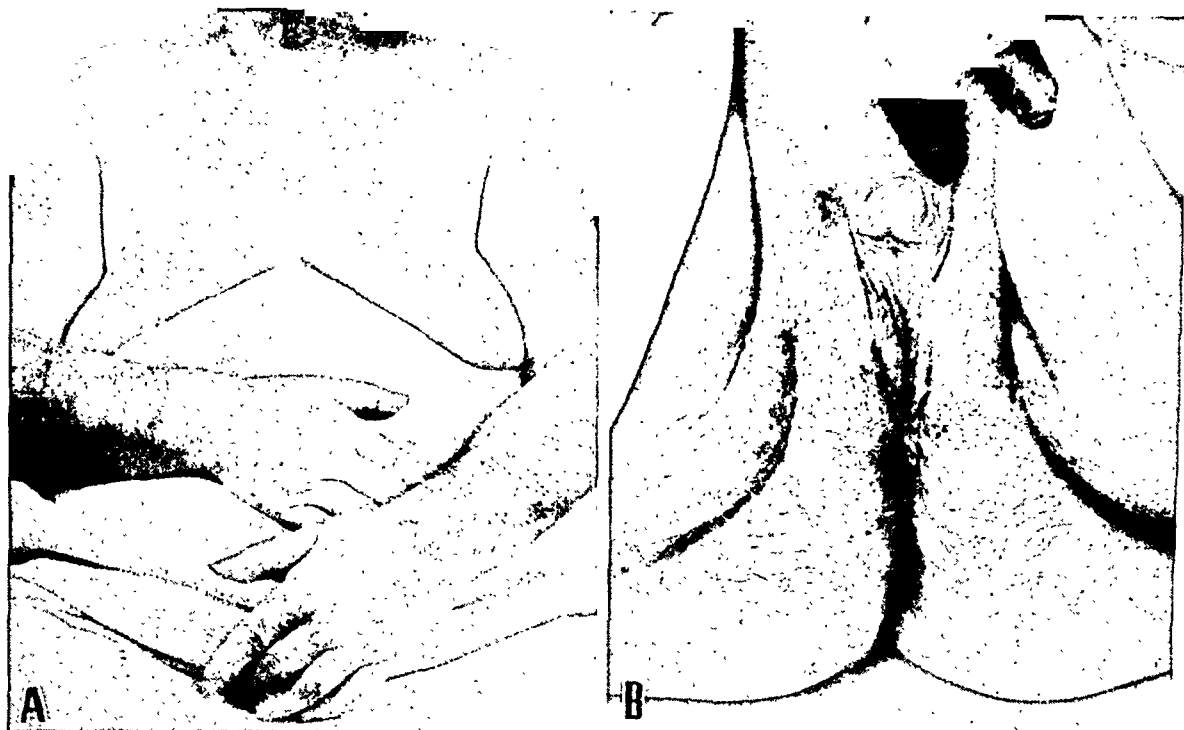


FIG. 2. A. Showing hirsutism of chest and arms. B. Showing hypertrophied clitoris $2\frac{1}{2}$ cm. in length and 1 cm. in diameter.

Laboratory examinations. Roentgenograms revealed a normal chest and skull. Examination of the renal areas by spot films, as well as intravenous pyelograms, showed nothing abnormal. The urine was normal and the Wassermann and Kahn tests were negative. The blood count on admission showed a hemoglobin of 16 grams, red blood cells 4.8 million, white blood cells 10,200 and a normal differential. The total nonprotein nitrogen was 28 mg. per cent, and the phenolsulphonphthalein urinary excretion was 50 per cent in one-half hour. Total serum protein was 6.9 Gm. per cent with albumen 5.3 grams and globulin 1.6 grams. The serum chloride level was 618 mg. per cent, cholesterol 185 mg. per cent and the cholesterol esters 59 mg. per cent. The Friedman test was negative. A twenty-four hour urine specimen contained 146.5 mEq. of sodium per liter and 32 mEq. of potassium per liter. The urinary excretion of 17-ketosteroids in twenty-four hours was 43 mg. Oral glucose tolerance and glucose-insulin tolerance tests showed a mild diabetic tendency and insulin resistance (Fig. 3). Basal metabolic rates on successive days were plus 8, plus 5, and plus 17 per cent. The histamine phosphate test showed no significant change in the blood pressure after the intravenous injection of 0.05 mg. histamine phosphate. The cold pressor test was normal.

Surgery. On February 13, 1947, a laparotomy was performed. The adrenal areas were palpated through a left upper rectus incision and it was thought no tumor was present.

The past history is almost a surgical calendar, with cholecystotomy at 36 years of age, cholecystectomy and appendectomy at 45. At 53 she had an exploratory laparotomy for fibroids of the uterus, but they were not excised. Instead, she was told, a section of bowel was removed. At 54-55 years a right iridectomy and cataract removal was done. Then at 59 years she underwent a left iridectomy and removal of a cataract. Mild diabetes, uterine fibroids and hypertension were confirmed during this latter hospitalization.

Physical examination revealed a healthy-appearing elderly white female, definitely obese and with heavy-lensed spectacles. Her temperature was normal, weight 217

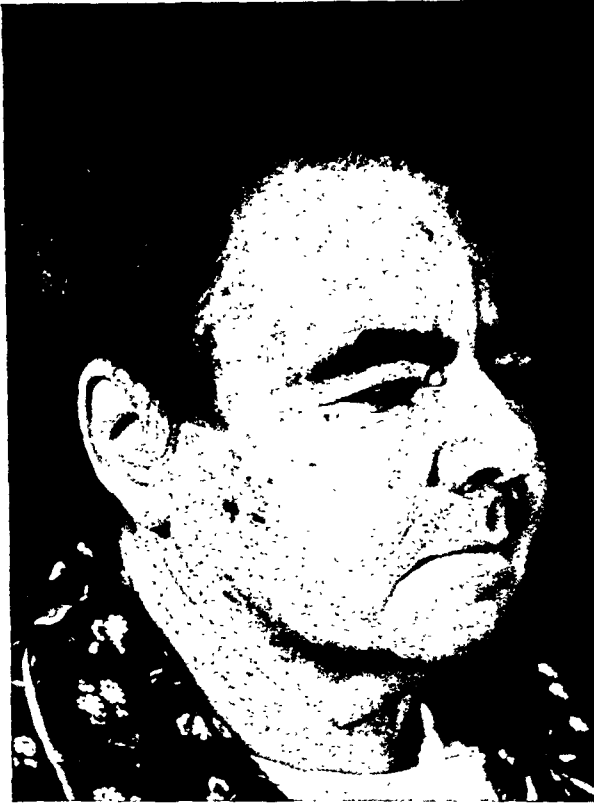


FIG. 1. Showing recently shaved beard and receding hairline.

pounds, pulse 76, and blood pressure 196/90. The scalp hair was noticeably thin and fine, with recession of the temporal hair line. There was coarse hair over the face, trunk, and arms with male distribution of the pubic hair (Figs. 1 and 2A). The distribution of fat was normal with the exception of a buffalo-type shoulder and neck contour. The skin was dry, coarse and brown. There were numerous pigmented elevated lesions, and raised waxy irregular lesions on the face, neck, upper trunk and arms. Eye, ear, nose, and throat examination revealed nothing abnormal except for bilateral iridectomies. The thyroid gland was not enlarged. The breasts were pendulous, and the abdomen showed an excess of adipose tissue. The chest was clear. The heart was not enlarged. There was a soft, short systolic murmur at the apex, not transmitted. The obese, pendulous abdomen

A huge fibroid uterus was found and the right ovary was replaced by a tumor mass about 3 inches in diameter. It had not invaded any of the surrounding tissues. The tumor was removed with some difficulty through a right McBurney incision. The patient's condition throughout the operation was good; however, the blood pressure dropped to 140 systolic during the last half hour.

Postoperatively the patient received a total of 365 mg. adrenal cortical extract (Percortin) over a six-day period, and 9 grams of sodium chloride daily in excess of her food intake for the first week. The blood pressure gradually increased during this period to preoperative levels. The temperature was normal at the fifth postoperative day. Laboratory examinations postoperatively and before discharge revealed a normal blood



FIG. 4. A. Tumor of ovary. Gross specimen intact showing marked lobulation. B. Cut section showing solid character of the tumor and lobulation produced by pseudo-capsules.

count and urine. The serum chloride level was 680 to 711 mg. per cent, serum sodium 127 mEq./liter and serum potassium 0.43 mEq./liter. The 17-ketosteroids were reported as 1.7 mg. in the twenty-four hour urine specimen. There was no significant change in the glucose-insulin tolerance test, the patient still showing an insulin resistance. At the time of discharge the patient was told to take stilbestrol 1 mg. daily three weeks out of every month.

Pathologically the specimen consisted of a tumor measuring 10 cm. in length, 8 cm. in greatest width, and 4.5 cm. in thickness (Fig. 4). Its external surface showed a capsule which was thin and did not appear to be intact over the entire specimen. The tumor mass was lobulated, and was homogeneous yellow in color except where the capsule was stained by blood. The lobular arrangement of the tumor was seen on cut section with the color again a bright yellow and soft in consistency. At one extremity of the tumor was an area softer than the remainder and somewhat red in color. This was thought to represent necrosis. The tumor weighed 130 grams.

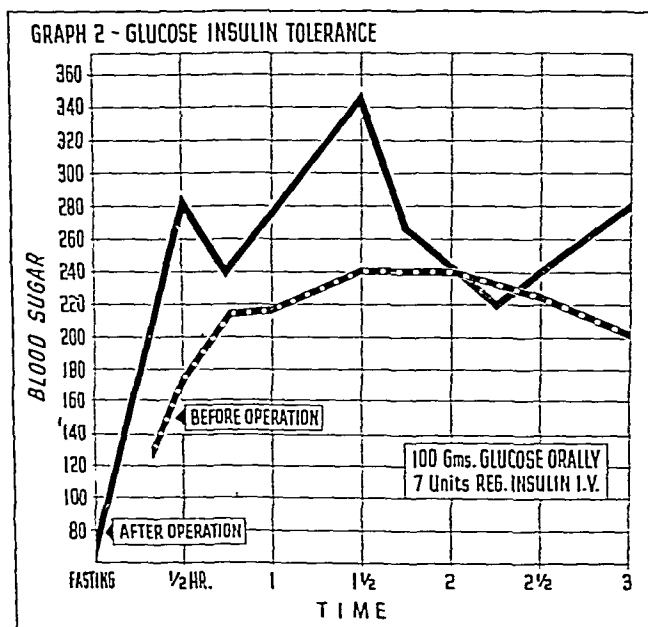
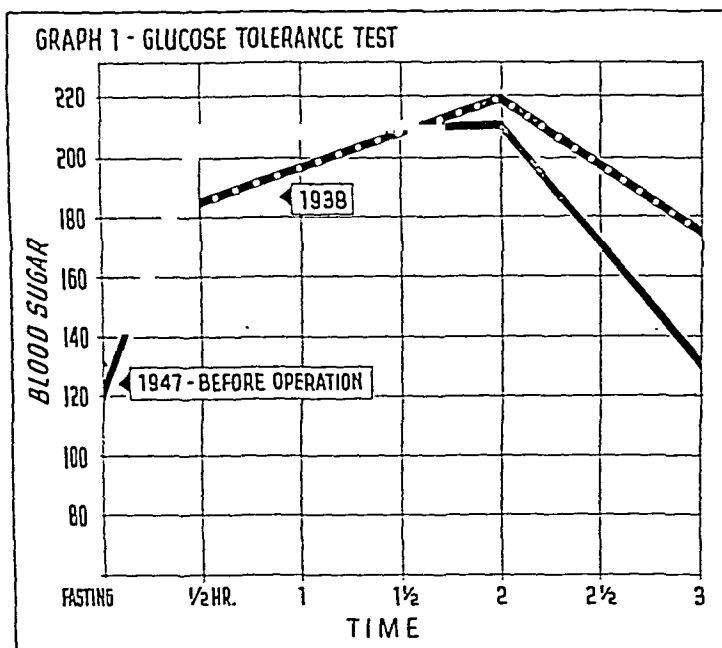


FIGURE 3

cells appeared to be arranged in strands or columns with a fine reticular stroma between the columns. The fine reticular stroma separating the surrounding cells appeared to be rather vascular with small papillary channels filled with red blood cells. The individual cells, in themselves, had a fine reticulum within the cytoplasm. With Sudan III stain, the cells were packed with fat (Fig. 6), and chemical analysis of the tissue showed it to be composed of approximately 85 per cent fat (8).

Postscript: A one year follow-up on this patient has shown a loss of most of the hair of body and extremities. The patient shaves three times weekly. Weight and blood pressure have remained as previously. The clitoris is grossly smaller and softer. The masculine characteristics have receded but have not disappeared. The patient had homologous serum jaundice $3\frac{1}{2}$ months after leaving the hospital.

DISCUSSION

The symptoms and signs associated with this tumor are beginning to be fairly well defined. However, the endocrine glands are so interrelated that the influence of the various hormones on one another has led to difficulty. As hormonal assays become more widely used it would seem that more distinct evaluation of clinical syndromes will be possible. In this type of tumor the symptoms as a rule are fairly well defined and consist of first defeminizing and later masculinizing signs and symptoms. This case was in a postmenopausal female who developed virilism and hirsutism. If Maxwell's (9) case is excluded as a typical masculinovoblastoma, this case is the only one occurring following the menopause. Voice changes were present but not to a marked degree. With Iverson's (10) table of clinical and morphologic features as a criterion, this patient fits in with those previously reported. The duration of the disease was four years; there was no weight loss or gain in the already obese individual; there was no pain referable to the tumor mass; and there was no abdominal swelling or palpable mass. Libido could not be evaluated in this unmarried woman. As mentioned, hirsutism was the prominent symptom. The clitoris was hypertrophied. The breasts were pendulous but there was no remarkable atrophy of breast tissue. The 17-ketosteroid value was elevated to 43 mg. This was estimated according to Robbie and Gibson's (11) rapid clinical method for extracting total urinary 17-ketosteroids. (The twenty-four hour specimen of urine, after hydrolysis with 10 per cent HCl, is extracted for ten minutes by refluxing with boiling CCl_4 and the hormone content is determined by the alkaline m-dinitrobenzene technique.) No estrogen or gonadotropic hormonal assays were done, as the medical institution was not equipped to do these laboratory determinations. Hypertension had existed a number of years prior to the onset of hirsutism. Eleven years before this hospitalization the patient was told she was a diabetic, and in 1940 she was given insulin for a period of months. The basal metabolic rate was within normal limits. These clinical and laboratory data fit in with the pathologic picture of a virilizing lipoid-cell tumor of the ovary.

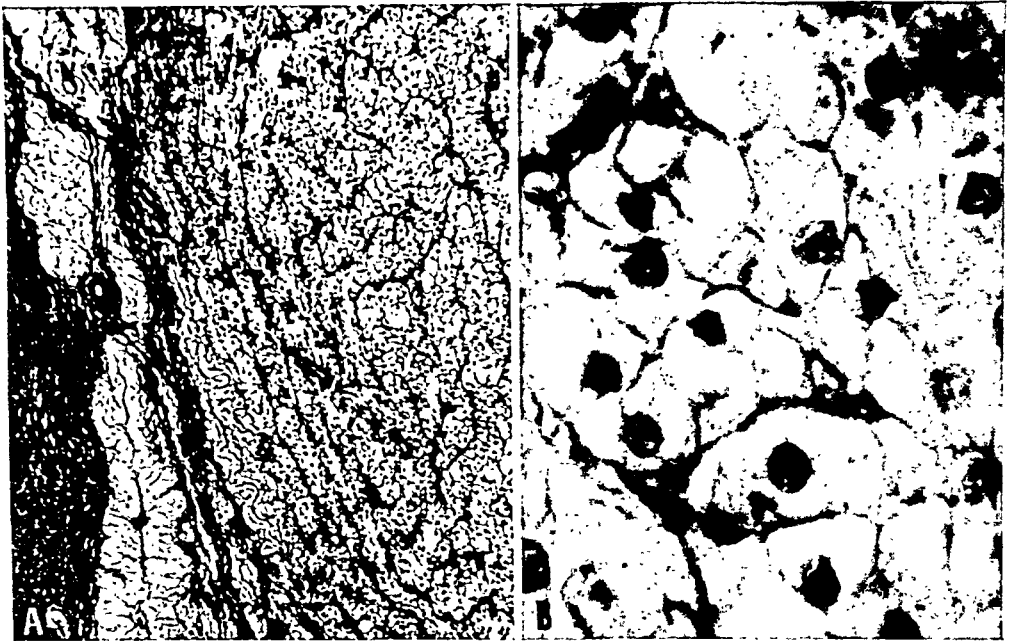


FIG. 5. A. Low power photomicrograph showing tumor on the right and ovarian tissue on the left. B. High power photomicrograph of tumor showing similarity to adrenal cortical cells.

Microscopic sections of the tumor revealed a tissue capsule surrounding tumor cells (Fig. 5). The capsule appeared normal for ovarian tissue. There were corpora albicantia present. The major portion of the tumor consisted of large polyhedral cells suggestive of lipid-containing cells or adrenal cortical cells with deeply staining granular nuclei. These

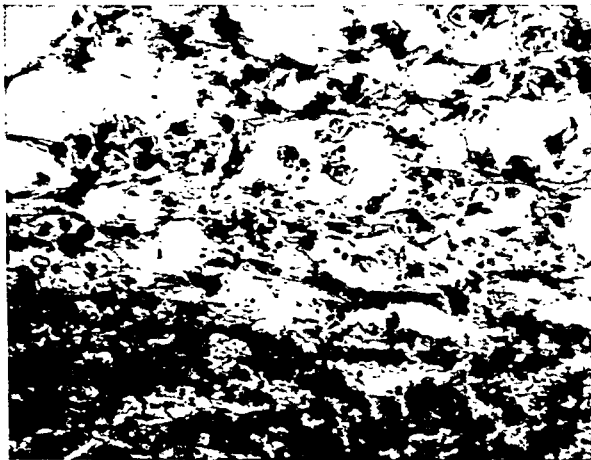


FIG. 6. Sudan III stain showing lipid content of cells.

PHEOCHROMOCYTOMA WITH HYPOTHALAMIC MANIFESTATIONS AND EXCESSIVE HYPERMETABOLISM

A CASE REPORT

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THE apparently hypothalamic origin of certain functional disturbances occurring in cases of pheochromocytoma has only rarely been emphasized. A case is reported here in which such manifestations were present as well as an extraordinarily high elevation of the basal metabolic rate and a sustained arterial hypertension.

CASE REPORT

M. T., a 28-year-old unmarried white female mill worker, was examined in Burlington, Vermont, on October 22, 1942. *Family history*: Parents, 5 sisters and 1 brother alive and well; 2 brothers died in early childhood of unknown causes. *Past history*: Scarlet fever, measles and mumps during childhood; otherwise always well. Menstruation began at age 14 and was always regular. She did not smoke tobacco nor drink alcoholic beverages.

Present illness:—During the year previous to examination she felt easily fatigued and lost weight (from 148 to 138 pounds) despite an excellent appetite and normal temperature. There was some palpitation and occasional shortness of breath, both on exercise and at rest; no dizziness; occasional moderate headache; and a slight degree of nervousness and irritability. A dusky red and bluish discoloration of her hands and legs developed during the year without being accompanied by any abnormal sensations. Once spontaneous blanching of one finger occurred, lasting twenty minutes. Her chief complaint was a continuous, intense perspiration from the waist up by day and night. Medication with Lugol's solution for five months had no effect.

Physical examination:—(1942). The patient was 5 ft. 6 in. tall, had a normal figure and healthy appearance. She was calm and cooperative. There was no conspicuous nervousness or motor restlessness, no exophthalmos or stare. The pupillary reflexes were normal. Some acne pustules were seen on the forehead, chin and back. The skin was very moist, especially so on the trunk and hands. There was an intensely dark, bluish-red, mottled discoloration of the lower legs and, to a lesser extent, of the hands. The discoloration faded markedly on elevation of the limbs and disappeared for a moment on local pressure. There was no edema. The deep reflexes were sluggish. There were no abnormal reflexes and no tremors. There was no enlargement of the thyroid. No glands were palpable.

The lungs appeared normal to percussion and auscultation. There was a constant

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At present we must rely mostly on evidence in the field of clinical and pathologic diagnosis for the classification of these tumors. The adrenal rest tumors of the ovary are very much alike in their microscopic picture but may vary in details enough to be confused with arrhenoblastoma(12). Our tissue has been submitted to the Gynecological Tumor Registry and has been classified by them as an adrenal rest tumor of the ovary (synonyms being masculinovoblastoma, hypernephroma of the ovary, adrenal cortical cell tumor of the ovary, and virilizing lipid cell tumor of the ovary).

SUMMARY

1. The clinical picture of a masculinizing syndrome in a postmenopausal woman, age 65, is presented and discussed.
2. A tumor mass which replaced the right ovary was removed.
3. Pathologically the tumor followed the picture described for an adrenal cortical cell tumor of the ovary.
4. This case is reported in an attempt to increase the knowledge of the clinical entity produced by a masculinizing tumor of the ovary, or masculinovoblastoma.

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tion of the systolic pressure of only 20 mm., and of the diastolic pressure of 10 mm. A benzodioxane test was not done, as this test had not yet been published before surgery was undertaken. The cold pressor test had no appreciable effect on the systolic and diastolic pressures. Sedation caused a maximum fall of the blood pressure to 150/90 mm. Postural fall of the blood pressure was repeatedly observed (*e.g.*, from 190/120 to 146/106) and was accompanied by cardiac acceleration (*e.g.*, from 108 to 140 beats per minute.)

Most striking was the blood pressure-depressing effect of slow hyperventilation in the recumbent position (Fig. 1). Both systolic and diastolic pressures fell to entirely normal levels within 15 minutes, while the heart rate remained unchanged.

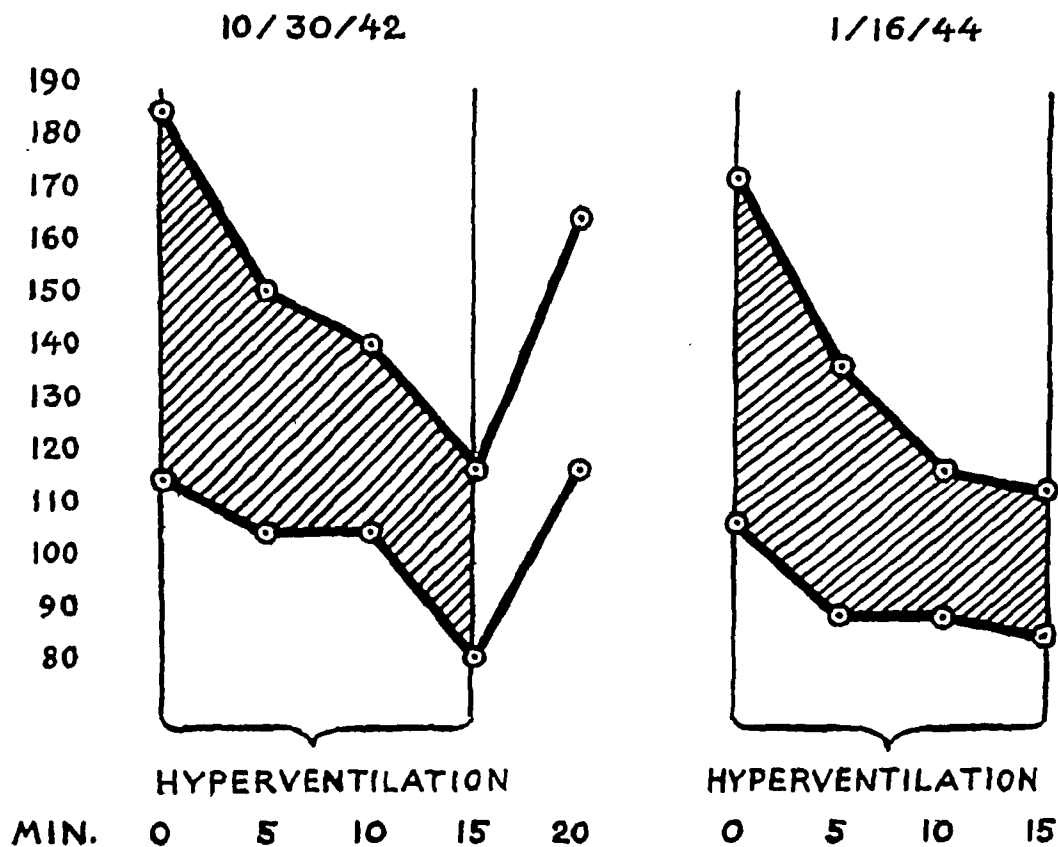


FIG. 1. Fall of systolic and diastolic blood pressure during fifteen minutes of hyperventilation, followed by the usual return toward the previous level after cessation of hyperventilation in the first experiment. The second experiment was interrupted.

Basal metabolic rate determinations were made twelve times between 1942 and 1945. During this period the B.M.R. varied between plus 69 per cent and plus 142 per cent, remaining mostly above plus 100 per cent. These readings were not affected by plugging the ears in order to eliminate a possible error through escape of oxygen. In 1947 the B.M.R. was considerably lower: plus 22, plus 33 and plus 36 per cent.

Urine specific gravity was 1014 to 1032. Albumin was 0 to 1.5 per cent (usually 0), sugar was 0 to 1.7 per cent (usually 0) and acetone was 0 to 3 plus (positive after twenty-four hours of a carbohydrate-free diet but also without such provocation). P.S.P. excretion in two hours was 30 per cent in 1944 and 51 per cent in 1947. Creatinine excretion in twenty-four hours was 1.9 Gm. (1944). In a 24-hour specimen the 17-ketosteroids were 3.5 mg. (1944, courtesy of Dr. H. Friedgood, Harvard Medical School).

tachycardia of about 120 beats per minute, a short systolic murmur over the apex and accentuation of the second aortic sound. The blood pressure was 220/130.

The abdomen was normal. The hymen was intact. The uterus was small and retroverted.

On the right eye ground, blurring and hemorrhages were seen in the lower temporal region of the disc margin and along the vessels extending into the fundus; the left eye showed papilledema and distended and tortuous veins. The ears were normal.

Initial laboratory and X-ray findings:—The electrocardiogram showed the following details; sinus rhythm, left axis deviation, PR 0.16 seconds, QRS 0.05 seconds, RT very slightly depressed in leads 1 and 2, T abnormally low in leads 1 and 2. On roentgen examination the heart appeared normal in size, shape and position; there was no indication of a substernal goiter. The pyelogram showed normal position of the kidneys and no abnormal shadows. A skull plate showed a normal sella turcica and no signs of increased intracranial pressure.

Examination of the blood showed a color index of 0.9, a count of 4,600,000 red cells, 12,100 white cells and a normal differential. The sedimentation rate was 17 mm. per hour. The Wassermann and Eagle tests were negative.

The basal metabolic rate was plus 69 per cent (one month earlier it had been plus 97 per cent).

Clinical course:—The patient felt subjectively well until the night of February 1, 1943, when she had a sudden attack of cough, dyspnea, choking, expectoration of frothy sputum, and cyanosis, which lasted for two hours and from which she recovered rapidly without a recurrence.

On June 5, 1943, she suddenly developed high fever (about 104° F.) which lasted for three days. She was restless, the heart rate was 140, but examination did not reveal any other abnormalities. Subsequently she felt exhausted for a month and she suffered from intense thirst which compelled her to drink 4 to 5 liters of water daily and to urinate six to eight times every night. At the same time, there was an irresistible craving for carbohydrates. She used to get up two or three times every night to eat a dishful of cereal each time. Menstruation ceased abruptly. Her weight dropped from 125 to 116 pounds. Within five months the polydipsia and polyphagia disappeared, menstruation reappeared, her weight returned to its former level and she resumed her work without further difficulties.

From November 1943 to January 1944, she received a daily dose of 0.6 Gm. of thiouracil. During this treatment the pulse rate decreased slightly (to about 110 per minute), but the basal metabolic rate and the blood pressure remained practically unchanged at a high level. The further clinical course from 1942 until her admission to the Massachusetts Memorial Hospital in Boston in 1947 was uneventful, except for a miscarriage three months after her marriage in 1946.

During these five years she was frequently examined, however, and the following findings were obtained:

Blood Pressure: fluctuated between 146 and 220 systolic and 106 and 130 diastolic, remaining mostly at the upper levels. Physical exercise was accompanied by a fall of the systolic and diastolic pressures, *e.g.*, from 146/102 to 132/0. The pulse rate rose simultaneously from 120 to 160 per minute. Subcutaneous injections of 0.1, 0.2 and 0.4 mg. of adrenalin did not affect the blood pressure level; intravenous injection of 0.2 mg. caused a sudden rise from 170/100 to 260/140, followed by a return to normal within four minutes. Intramuscular injection of 0.05 mg. of histamine was followed by an elev-

prominent nuclear membranes; in the smaller cells the nuclei were round or ovoid and vesicular. No mitoses were seen.

Bio-assay of the tumor was carried out by Dr. O. Kraye of the Harvard Medical School with tests on blood pressure and isolated gut. It revealed that tumor extracts exerted activities corresponding to an epinephrine equivalent of 0.3 mg. per gram of fresh tissue.

Kidney tissue removed during the operation showed the following histologic details: "occasional arterioles and pre-arterioles present slight intimal sclerosis and/or hyaline deposition, and a few arteries show slight to moderate intimal sclerosis. There are three hyalinized glomeruli. The other glomeruli are normal or show only slight hyaline thickening of capillary basement membranes." Diagnosis: nephrosclerosis, grade 1.

COMMENT

The case described here appears significant because of its unusual symptomatology and because of its analogies with the syndrome of essential hypertension, which emphasize the possibility that both clinical entities have closely related neurohormonal pathogenic mechanisms in common, as suggested by various investigators (1, 2, 3).

Manifestations pointing toward a participating diencephalic disturbance, not infrequently observed in cases of pheochromocytoma, were the following: hyperhidrosis, hyperthermia, polydipsia, polyuria, polyphagia and amenorrhea. Furthermore, there was extreme hypermetabolism of non-thyrotoxic origin with no response to thiouracil and prompt normalization after removal of the pheochromocytoma. The latter phenomenon may have been due, to some extent, to the experimentally demonstrated (4) role of

TABLE 1

Highest B.M.R. readings in hypertensive* patients in whom no pheochromocytoma was suspected or diagnosed, as reported by various authors

B.M.R.	Reference	B.M.R.	Reference	B.M.R.	Reference
+20%†	(6)	+38%	(14)	+55%	(22)
+20%	(7)	+40%	(15)	+77%	(23)
+20%	(8)	+41%	(16)	+80%	(24)
+28%	(9)	+50%	(17)	+82%	(25)
+31%	(10)	+50%	(18)	+84%	(26)
+33%	(11)	+52%	(19)	+122%	(27)
+35%	(12)	+55%	(20)	+160%‡	(28)
+36%	(13)	+55%	(21)		

* No cases with cardiac decompensation or uremia are included in the above compilation.

† In 3.4% out of 170 cases with hypertension

‡ Postencephalitic hypertension.

Blood chemistry: The blood sugar level (fasting) was 95 to 118 mg. per cent. A glucose tolerance curve was normal in 1944 and showed mild diabetes in 1947. N.P.N. was 27 to 40 mg. per cent, creatinine 2 to 6 mg. per cent, cholesterol 233 to 320 mg. per cent, calcium 9.9 to 12.1 mg. per cent, phosphorus 3 to 6 mg. per cent, phosphatase 2.1 units, total iodine 11.3 micrograms per cent (courtesy Mr. H. J. Perkin, Lahey Clinic, Boston), serum proteins 7.2 Gm. per cent, chlorides 104 milliequivalents per liter and alkali reserve 28.6 milliequivalents. Prothrombine time was normal. Adreno-sympathetic catechol compounds in the blood were within normal limits on two occasions (colorimetrically assayed). On the isolated frog heart the serum caused a moderate increase of the amplitude.

Electrocardiogram: Unchanged from 1942 to 1945. In 1947: T1 upright, T2 diphasic (— +), T3 inverted and T upright in CF4, 5. Normal axis position.

Heart size: (X-ray) 12.5 cm. in 1942, 13.1 cm. in 1947. The inner diameter of the chest was 27.5 cm.

Electroencephalogram: (Dr. Toman) Normal with moderately strong alpha rhythm.

Eye grounds: Somewhat improved after 1944. In 1947: grade 2 hypertensive and grade 1 sclerotic changes.

Surgery: After repeated and prolonged persuasion, the patient finally entered the Massachusetts Memorial Hospital in Boston in 1947 and on April 24 of that year the right sympathetic trunk was removed from D-10 to L-1 inclusive and the great splanchnic from just above the celiac ganglion upward to the level of D-10. On the left side a tumor of the adrenal gland was found and resected.

At the time of the removal of the tumor the blood pressure dropped precipitously and this critical condition remained the same following the operation. It necessitated the infusion of a total of 54 mg. of adrenalin within seventy-two hours in addition to substantial amounts of blood and plasma. On the third postoperative day the blood pressure became stabilized. For six days she remained mildly disoriented but on the fifteenth postoperative day she was discharged in good condition. At that time the blood pressure ranged from 110/72 to 135/100 and the pulse rate from 78 to 100.

When she was seen one month later in Burlington, she felt perfectly well. The discoloration of legs and hands and the excessive perspiration had disappeared. The blood pressure was 104 to 116 systolic, 78 to 80 diastolic and the pulse rate was 81 to 144 (the latter only in the standing position). The electrocardiogram was normal. The urine was normal. P.S.P. excretion was now 70 per cent in two hours. The B.M.R. was plus 2 per cent. According to recent reports she is still in excellent health. She gave birth to a 9 lb. 6 oz. child on Jan. 27, 1949.

Pathology of the tumor:—(Dr. J. Diamond) The adrenal tumor measured 4.3 cm. in length, 3.1 cm. in width and 2.5 cm. in thickness. It weighed 20.7 grams with a minute remnant of uninvolved adrenal tissue at either pole. It showed thin yellow patches of cortical tissue beneath the capsule and small areas of hemorrhage. The medulla was reddish-gray and soft. In the mid-portion there was a small cyst which contained thin, red fluid.

Microscopically the tumor was identified as a pheochromocytoma, consisting of adrenal medullary cells arranged in compact cylindrical cords which were bounded by fibrocollagen. The cells varied from a small round form to a moderate sized fusiform shape, interspersed with many extremely large cells whose boundaries were indistinct. The cytoplasm was eosinophilic in all types of cells, finely granular with a fine reticulation and occasional vacuolization. The larger cells contained very large nuclei with

SUMMARY

A case of pheochromocytoma is reported which showed the following peculiarities: sustained arterial hypertension; tachycardia; hyperhidrosis of the trunk; fall of the blood pressure to normal levels during hyperventilation; hypermetabolism with a basal metabolic rate as high as plus 142 per cent; hypothalamic manifestations (hyperthermia, polyuria, polydipsia, polyphagia); occasional glycosuria and acetonuria; acrocyanosis; vascular retinopathy and impaired kidney function.

All pathologic phenomena disappeared promptly after removal of the adrenal tumor.

Certain analogies of symptoms and neurohormonal pathogenic mechanisms with those of essential hypertension are briefly discussed.

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the diencephalon in the regulation of general oxygen consumption. On the other hand, there exists an extensive literature, recently reviewed by B. Kisch (5), concerning the catalytic, oxygen-consumption-stimulating function of epinephrine and of its quinoid derivative (omega, adrenochrome) which makes it probable that the sometimes very marked elevations of the B.M.R. in cases of pheochromocytoma are caused by the presence of abnormal amounts of these and related substances.

Since high basal metabolic rates are frequently found both in cases of pheochromocytoma-induced and of essential hypertension (Table 1), this peculiarity can be listed together with the many metabolic, cardiovascular, electrocardiographic, renal, cerebral, neurovegetative and ocular abnormalities which occur in both conditions, even though in varying frequency and distribution.

The phenomenon of a marked fall of the blood pressure during slow hyperventilation, which was particularly pronounced in the case reported here (Fig. 1) has been interpreted as a criterion of abnormal central vasomotor irritability (29, 30, 31). It, too, is not specific for pheochromocytoma-induced hypertension but constitutes a common finding in essential hypertension (29-39).

Indications for a fundamental significance of hyperactive sympathomimetic neurohormones in the pathogenic mechanism of pheochromocytoma-induced as well as of essential hypertension are steadily increasing (1, 2, 3). In the case of pheochromocytomas, epinephrine and nor-epinephrine (40), discharged from one primary source (the tumor) into the general circulation, are the responsible agents. In essential hypertension, on the other hand, nor-epinephrine (sympathin) (41-44), which is discharged from a multitude of postganglionic fibers into their respective cardiovascular effector cells (45-48), must be suspected as an important causative factor (1). Whether an epinephrine-like vasopressor amine (encephalin) which has recently been isolated from the brain (49) is involved in the hypothalamic features, occurring both in pheochromocytoma-induced hypertension and in essential hypertension (3, 11, 25), remains to be investigated.

Cases such as the one described above in which all cardiovascular, neurovegetative, metabolic, renal and other abnormalities disappear promptly after surgical intervention, emphasize the value of a timely elimination of the epinephrine-nor-epinephrine-discharging tumor. Similar results, achieved through surgical removal of nor-epinephrine-discharging sympathetic nervous tissue in essential hypertension (48, 50, 51), serve as a further indication of pathogenic similarities which are a challenge for more systematic study of this field.

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The Association for the Study of
Internal Secretions
Request for Biographical Data for
New Roster

The membership roll of the Association is being revised in preparation for the issuance of a new Roster as of December 31, 1949. *Copy for this must be in the hands of the printer not later than October 1, 1949.*

Return postal cards are being mailed to members requesting the following information:

Name.....	Degrees.....
Mailing Address.....	
.....	
Teaching or Research Position:.....	
.....	
.....	
If in active practice of medicine, specialty:.....	
.....	

Members are urged to complete and return the card immediately and to keep the Secretary informed at all times of any change in the above.

HENRY H. TURNER, M.D.
Secretary-Treasurer
1200 North Walker Street
Oklahoma City 3, Oklahoma

The 1950 Meeting of the Association
for the Study of Internal Secretions

The next Annual Meeting of the Association will be held in San Francisco, June 23 and 24, 1950. The hotel in which we will meet has not yet been determined because of the uncertainty of AMA commitments, but it will be announced in an early issue of the Journal. Members are urged to make their hotel reservations as soon as possible.

Letter to the Editor

HYPOGLYCEMIA IN THE EARLY PHASE OF ADRENOCORTICAL CARCINOMA

Comments on the paper "Corticoadrenal Tumor with Hypoglycemic Syndrome, Goiter, Gynecomastia and Hepatosplenomegaly" by Juan José Staffieri, Oscar Cames and José M. Cid. (*J. Clin. Endocrinol.* 9: 255-267 (March) 1949).

TO THE EDITOR:

AT the meeting of the Association of American Physicians in 1946 (discussion of paper by Thorn, G. W.: *Tr. A. Am. Physicians* 54: 119, 1946), I communicated the incidence of hypoglycemia in 3 patients (2 females, 1 male) with adrenocortical carcinoma. All 3 cases were confirmed by biopsies. In one of the females, the hypoglycemia was first recognized in a state of hypoglycemic attack with unconsciousness. Slight hyperglycemia, however, developed in later phases of the disease in all 3 patients. These patients exhibited disfiguring puffiness of their faces and severe eruptions of acne. The females had only slight virilism. The male patient showed gynecomastia, which also persisted after successful removal of the tumor (Dr. Kenneth Thompson). The male patient is still alive three and one-half years after operation. Both females died. They had large livers with metastasis of the tumor. In 2 cases (1 female and 1 male) the 11-oxysteroids in the urine were found increased (Dr. Nathan Talbot, Boston): 7.6 mg. per cent and 1.3 mg. per cent respectively (normal 0.1-0.3 mg. per cent).

S. J. THANNHAUSER, M.D., Ph.D.
May 2, 1949.

Joseph H. Pratt Diagnostic Hospital,
30 Bennet Street,
Boston, Massachusetts.

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or pre-clinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for \$1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to \$1,800.

* * * * *

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950*.

The 1950 Meeting of the American Goiter Association

The next meeting of the American Goiter Association will be held at the Hotel Shamrock, Houston, Texas, March 9, 10, and 11, 1949. It is recommended that all physicians wishing to attend make their hotel reservations early.

The 1950 Awards of the Association for the Study of Internal Secretions

FELLOWSHIPS

THE AYERST, MCKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Lawrence Wilson.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed \$2,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study. He must indicate one or more institutions where the proposed program will be followed. He must submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AWARDS

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of \$1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945

The ketosteroids were determined in 24-hour urine specimens by an adaptation of the method of Robbie and Gibson previously described (18, 19). Specimens were collected once a week for a sufficiently long period of time to cover a complete menstrual cycle.

Figure 1 represents weekly values of ketosteroid excretion in 2 normal young women in relation to the day of the menstrual cycles. The limits of the normal female excretion range as found in this laboratory (5 to 18 milligrams per 24 hours) are indicated by horizontal broken lines. The excretion pattern is in agreement with the observation mentioned above, that there is apparently no pronounced change in the excretion level at any particular phase of the cycle.

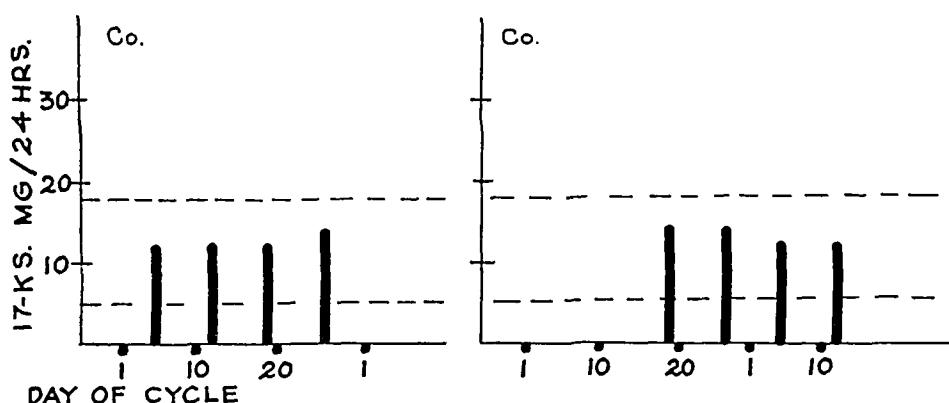


FIG. 1. Ketosteroid excretion in normal women.

Figure 2 represents in the same way the ketosteroid excretion in 9 cases of idiopathic hirsutism in which a complete record could be obtained. It is evident that a) the excretion level is in general well above the normal range, but may at times drop to normal values, b) there is a cyclic variation associated with the menstrual cycle, and c) the maximum excretion values are found at the approximate time of ovulation. The excretion levels satisfy the varying qualifications in the literature, ranging from normal to elevated, depending on the phase of the cycle during which the determination is made.

The cyclic variation pattern can be regarded as originating in one of two ways: 1) the total excretion of the adrenal cortex of these patients is subject to cyclic change, or 2) the output of the adrenal cortex does not vary appreciably but is augmented by excretion from another source which is subject to ovarian change.

If it was thought useful to compare these findings with the ketosteroid excretion pattern of normal female rats in relation to the estrus cycle. To that end, total daily ketosteroid determinations were made on the urine of isolated adult virgin female albino rats with established regular estrus cycles.

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THE EXCRETION OF 17-KETOSTEROIDS IN IDIOPATHIC HIRSUTISM

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of Medicine, San Francisco, California*

THE observations which have been published on the excretion of urinary 17-ketosteroids in normal women show that, though fluctuations in the excretion level occur, no systematic relation can be demonstrated between these fluctuations and the different phases of the menstrual cycle (1-11). The level of ketosteroid excretion during the menstrual cycle is on the whole remarkably constant, in contrast to that of other substances of hormonal origin.

Ketosteroid excretion in hirsutism has been reported as high or very high, only when the condition is associated with the presence of a masculinizing tumor (adrenal or ovarian). In other cases of hirsutism the excretion range has been described as "within the normal range," "tending toward the male range of excretion," or as "moderately elevated" (3, 9, 12-17).

The following report deals with the 17-ketosteroid excretion of 9 patients complaining of increased growth of facial and body hair. The patients ranged in age from 21 to 30 years; 4 were single, 5 were or had been married and none had children. Menstruation in all patients was described as regular or essentially regular. The complaint of increased hair growth extended over periods varying from two to four years. Physical examination revealed no gross change in organs or other cause of this condition.

Received for publication January 25, 1949.

* Agnes Lemme Schilling Fellow in Obstetrics and Gynecology.

estrus can be ascribed to stimulation of this organ, directly or indirectly, by the animal's estrogens.

From the similarity of the excretion patterns the conclusion can be tentatively advanced that the increased ketosteroid excretion in idiopathic hirsutism is likewise the result of a stimulation of the adrenal cortex by ovarian hormones and that the condition of these patients is due to a

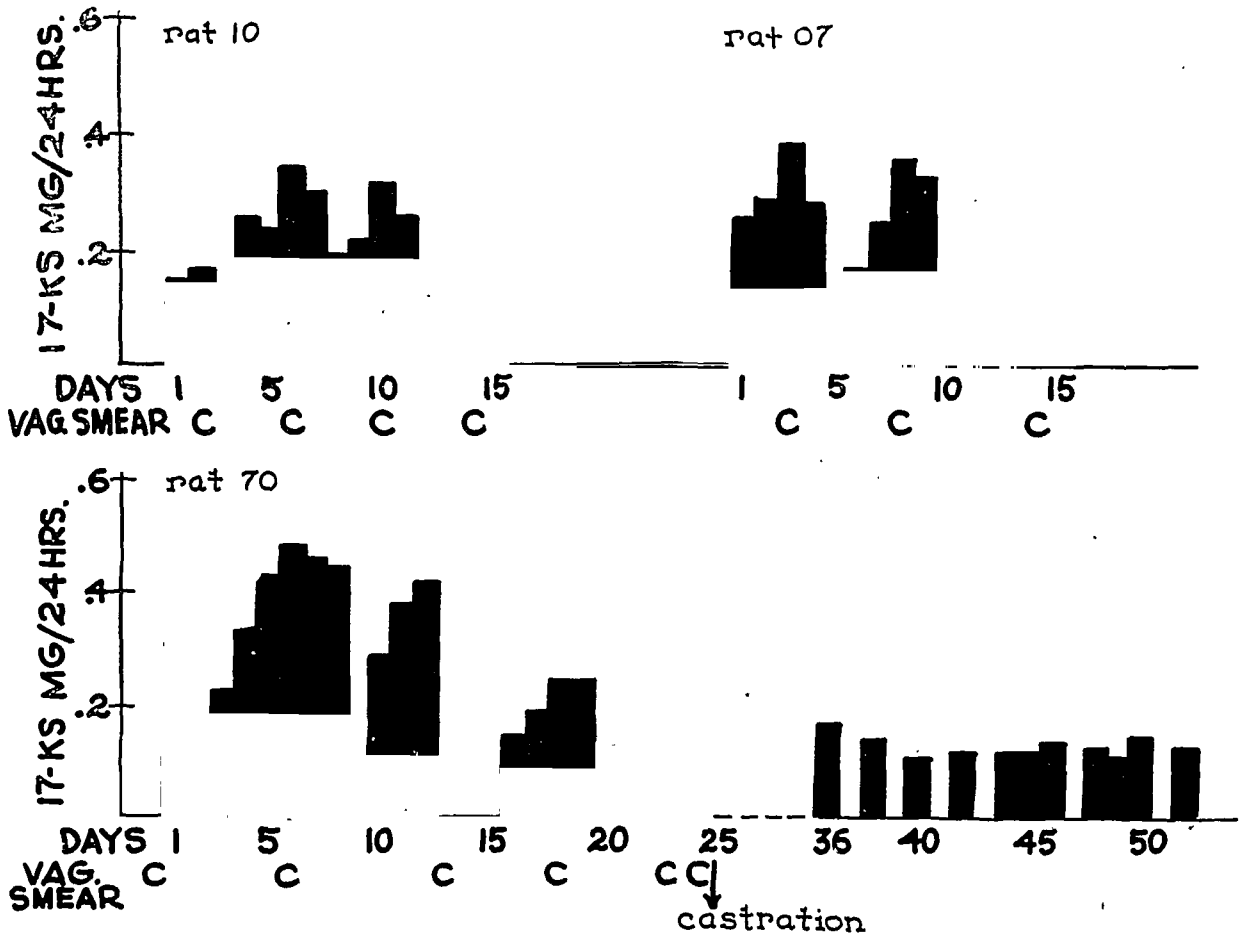


FIG. 3. Ketosteroid excretion in normal female rats. (C indicates a vaginal smear showing cornified cells.)

sensitivity of the adrenal cortex which is absent or imperceptibly small in normal women.

It should however be borne in mind that it may not be the cyclic variation of the ketosteroid excretion of the hirsute patient which requires an explanation but rather the relative constancy of the excretion during the menstrual cycle of the normal woman. It is possible that in the latter a stimulation of the adrenal cortex is balanced by a simultaneous inhibition of the adrenotropic activity of the pituitary gland. The possibility of such an inhibition seems indicated by observations of Ross and Hamblen

The results are represented in Figure 3. It is evident that a pronounced variation of ketosteroid excretion exists, and that the maximum excretion occurs at the time of vaginal cornification, that is, at mid-estrus, corresponding to the time of ovulation in woman. The excretion pattern of these normal female rats is therefore similar, not to that of normal women, but to that of the hirsute patients.

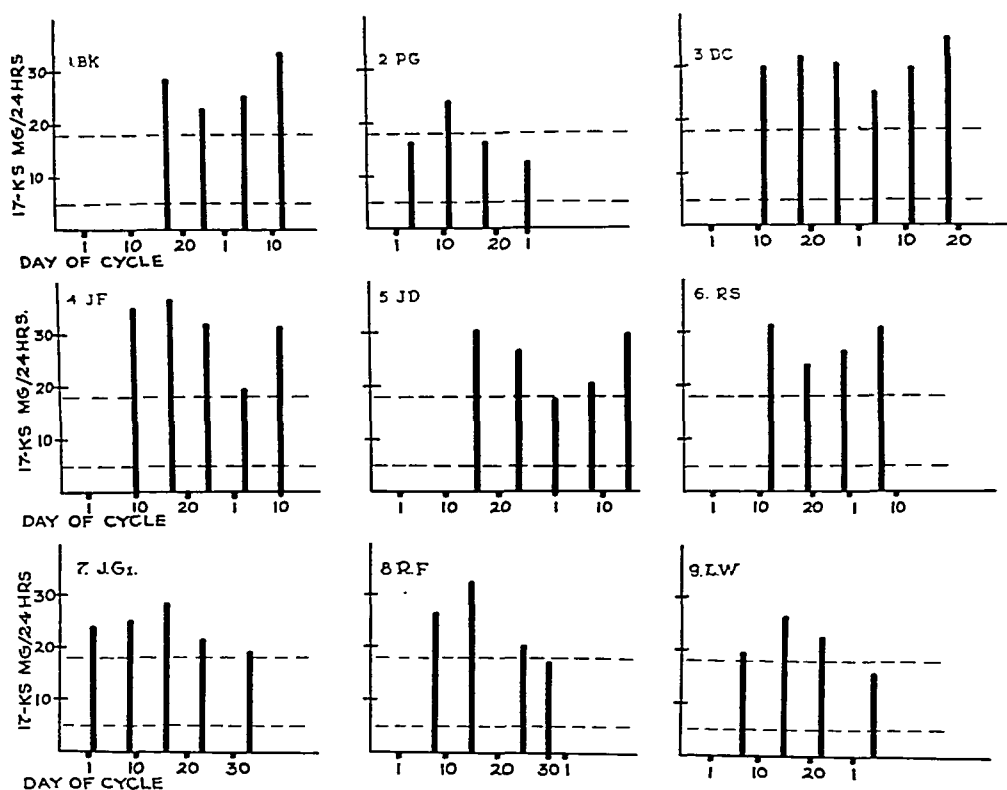


FIG. 2. Ketosteroid excretion in idiopathic hirsutism.

After ovariectomy of the rat the cyclic variation of the excretion level ceased and the level dropped to minimal values. After adrenalectomy of an otherwise intact rat, the ketosteroid excretion dropped to below measurable levels.

This pattern is evidently the chemical expression of the changes in the adrenal gland previously described by Andersen and Kennedy and by others (20, 21) who observed that the weight of the adrenal cortex of the female rat varies with the estrus cycle, reaching its highest value at estrus, and that in spayed animals the increase in weight can be brought about by injections of estrogen. This change in the adrenal cortex at the time of

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(22-24) of a temporary rise of ketosteroid levels after cessation of ovarian activity. Lack of data on adrenotropic hormone output in relation to the phases of the menstrual cycle does not make further speculation on this point profitable.

SUMMARY

Ketosteroid excretion levels in 9 cases of idiopathic hirsutism, measured at weekly intervals during complete menstrual cycles, show a cyclic variation with a maximum excretion at the approximate time of ovulation, in contrast to the relative constancy of excretion in normal women. The maximum levels are invariably well above the upper limits of the normal excretion range.

A similar cyclic excretion pattern with a maximum at the time of estrus is observed in normal female rats in which variations in weight of the adrenal cortex with the estrus cycle were known to occur.

The conclusion is tentatively advanced that the condition of the hirsute patient is connected with a sensitivity of the adrenal cortex to ovarian hormones which is absent in normal women.

Acknowledgment

The assistance of members of the medical profession in providing the diagnoses and in making suitable specimens available is gratefully acknowledged.

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We also conducted a few brief studies with (Ca) 4-n-propyl-6-oxy-pyrimidyl-2-mercaptoacetic acid,¹ which for convenience we refer to as 2-(acetic acid) propylthiouracil.

The formulas of three of these compounds are shown in Figure 1.

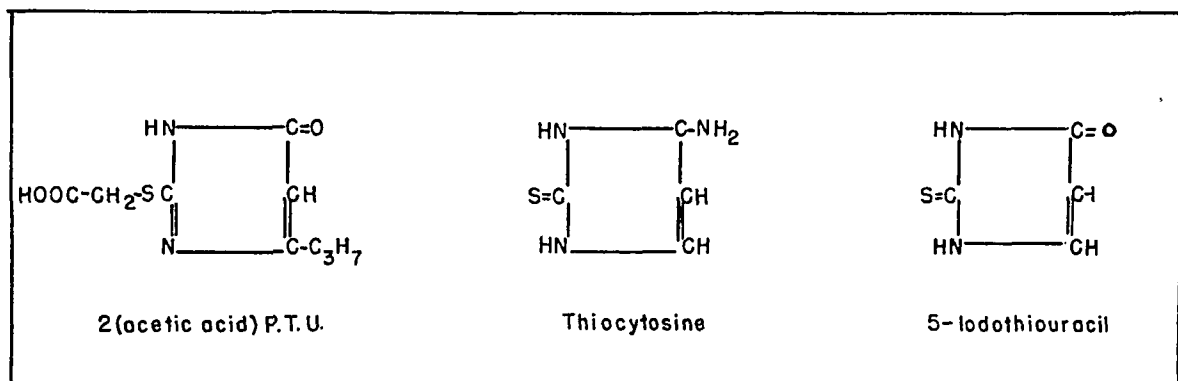


FIG. 1.

5-Iodo-2-Thiouracil

(a) *Effect upon thyroid size and concentration of non-radioiodine in rats.*

Barrett *et al.* (2) found that 5-iodo-2-thiouracil injected subcutaneously in rats was approximately one-half to one-third as effective as 2-thiouracil, judged by goitrogenesis or decreased iodine in the thyroid. When iodothiouracil was fed at a 50 per cent higher level, on a molar basis, than was thiouracil, it was found that the former was about one-half as effective as thiouracil in decreasing the (total) iodine content of the thyroid gland and less than 50 per cent as effective in goitrogenesis. When equimolar quantities of potassium iodide and thiouracil were given, the degree of goitrogenesis was greater than in the group which received iodothiouracil, and the iodine concentration in the thyroid was less (3).

(b) *Effect upon concentration of radioiodine in the thyroid of rats.*

Two experiments were conducted in rats, using radioactive iodine (I^{131}), to test the effect of iodothiouracil upon the uptake and release of the isotope by the thyroid. Its effects were compared with that of an equivalent amount of iodide alone, of thiouracil alone, and the two in combination. All of the rats used were male, Sprague-Dawley strain, and were aged approximately 5 months. Seven rats served as controls and there were 4 or 5 in each of the other groups. Each was fasted for fifteen hours and received 50 microcuries of radioiodine in 1 cc. of saline subcutaneously at the time indicated in the following paragraphs.

In the first experiment the antithyroid compound was administered two

THE ANTITHYROID ACTION OF 5-IODOTHIOURACIL, 6-METHYL-5-IODOTHIOURACIL, THIOCYTOSINE AND (Ca) 4-n-PROPYL-6-OXYPYRIMIDYL-2-MERCAPTOACETIC ACID*

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AN interest in the antithyroid activity of iodothiouracil and thiocytosine was expressed in an earlier report (1) of observations upon various goitrogenic agents, but neither compound was available.

It appeared possible that iodination of thiouracil might increase the concentration of the compound in the thyroid, due to the avidity of the gland for iodine. Such a selective action would render it unnecessary to expose the other tissues to as much thiouracil as is usually the case. It was thought that even though the compound did not act in this manner it might possess an advantage over thiouracil, either in possessing a stronger antithyroid action or a less toxic one. Interest in the compound persisted in spite of the fact that the antithyroid action of two iodinated aminobenzoic acids depended solely upon the effects of the iodine which they contained (1).

Dr. H. W. Barrett and associates (2) recently synthesized 5-iodo-2-thiouracil and 6-methyl-5-iodo-2-thiouracil and he and Dr. F. X. Gassner very kindly afforded us the opportunity to study these compounds.

Having studied sulfur derivatives of uracil and thymine we were interested in investigating thiocytosine.¹ It was thought that the variations in the intracellular distribution of the three natural pyrimidines might produce significantly different effects.

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¹ We are very grateful to Dr. Augustus Gibson of Merck and Co., Rahway, N. J., for our supply of thiocytosine and to Dr. Ernst Oppenheimer for the (Ca) 4-n-propyl-6-oxypyrimidyl-2-mercaptoacetic acid.

groups receiving iodine than in the group to which thiouracil alone was given. It was slightly greater in the ones to which were administered potassium iodide than in the ones given iodothiouracil. It is difficult to know just how much of the antagonizing effect of iodothiouracil is to be attributed to the compound as such, how much to iodide and thiouracil which may have been liberated from iodothiouracil, and how much to various combinations of these compounds.

When the antithyroid compounds were administered two hours after the radioiodine larger quantities of isotopes accumulated in the thyroid than when they were given two hours before the tracer substance. However, some antagonism was exhibited by each of the compounds in each experiment. There was less difference in the degree of effects of the compounds when they were administered after the isotope.

(c) *Effect upon discharge of radioiodine from the thyroid of normal subjects.*

Doctors Astwood and Stanley kindly conducted studies with iodothiouracil similar to the ones they used in testing the antithyroid effect of many compounds (4). The assay depends upon modification of a predictable pattern of accumulation of radioactive iodine by the thyroid gland as detected by serial counts with an externally placed Geiger-Müller counter. As compared with thiouracil the action of iodothiouracil was delayed, requiring six hours to be manifested. Dr. Astwood suggested that the delayed reaction might be associated with a slow liberation of iodine from the iodinated thiouracil.

(d) *Distribution of iodothiouracil throughout the body.*

Studies conducted to determine the distribution of iodothiouracil throughout the body were unsatisfactory because of the failure of iodothiouracil to give a satisfactory color reaction with Grote's reagent.

(e) *Effect in patients with thyrotoxicosis.*

Twenty-nine unselected patients with thyrotoxicosis were given 40 courses of therapy with iodothiouracil. Twelve of the patients were hospitalized while therapy was conducted. White blood cell counts were performed upon these individuals from one to two times weekly. The other patients were ambulatory, and were observed at weekly intervals; white blood cell counts were conducted in these whenever any infection was present.

From 60 to 300 mg. was given daily in equally divided doses at approximately eight-hour intervals; the usual dose was 150 mg. per day.

The response varied considerably; some patients with severe thyrotoxicosis had a complete remission within ten days, whereas others never had

hours before radioiodine, whereas in the second the order of administration was reversed. Each of the antithyroid compounds was administered intragastrically in 5 cc. of water. Each rat in the groups receiving iodothiouracil was given 50 mg. The ones in the groups given potassium iodide received the same amount of iodine as is present in 50 mg. of iodothiouracil. Likewise, the ones given thiouracil alone received the same amount

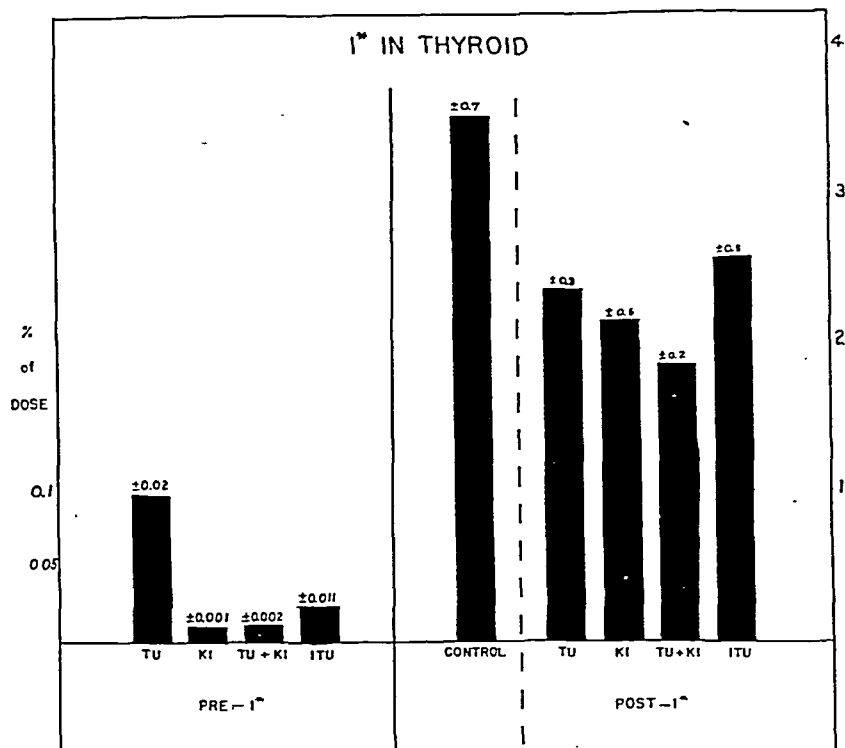


FIG. 2. The groups designated as pre-I* received the antithyroid compounds two hours before radioiodine, whereas the ones listed as post-I* were given the isotope two hours before the antithyroid compounds. The standard deviation from the mean is indicated.

as is present in 50 mg. of iodothiouracil. All rats were killed four hours after receiving radioiodine. The thyroid gland was removed, weighed quickly, ashed with potassium hydroxide and the percentage of the administered dose of radioiodine which was present in the thyroid was determined.

The results of the two experiments are presented in Figure 2. It may be observed that when any of the compounds was administered two hours before the radioiodine, the amount of the latter concentrating in the thyroid was markedly lowered. The antagonism was greater in each of the

TABLE 1. PATIENTS TREATED WITH IODOTHIOURACIL.

Patient No.	B. M. R. %			Maximal total daily dosage (mg.)
	Initial	Maximal response	Days required	
#103	+28	+ 9	9	100
#112	+25	0	9	100
	+39	- 8	8	100
	+25	+11	9	150
#113	+74	+20	6	150
#114	+24	+ 8	6	150
#115	+29	- 1	9	100
	+32	+10	14	150
#116	+29	+14	7	100
#117	+38	+14	24	100
#118	+50	+18	7	150
	+45	+12	20	150
#121	+26	+ 7	12	150
#122	+41	+27	15	150
	+31	+23	28	150
#123	+30	+11	7	100
	+27	+17	18	150
#125	+60	+ 1	14	150
#126	+59	+ 3	10	150
	+60	+26	28	200
#127	+25	+ 7	7	150
	+17	+ 8	15	90
#128	+45	+15	13	150
#129	+50	+11	7	100
	+54	+ 7	30	150

a complete recovery (see Table 1). Of 15 subjects with a maximal response in ten days, the average decrease in basal metabolic rate per day was approximately 4 per cent; of 21 with a maximal response in fifteen days, the average decrease was 3 per cent. Means and associates (5) found that with iodide therapy alone the average fall in metabolic rate was 3.6 points per day. The individuals who had not experienced a complete remission within fifteen days never had one, although some of them were treated for several additional weeks. Eleven patients never became completely free of thyrotoxicosis, although each subject showed some improvement. None of the subjects who became euthyroid experienced a relapse under continued therapy, but in a few individuals with incomplete responses the thyrotoxicosis became less well controlled, although the dosage was increased. In no instance was there a complete relapse while the patient was continuing therapy. However, following cessation of therapy a relapse was apparent within one or two weeks. Nine patients had two or more courses of therapy. In three, the response was not as good with the second course as with the first, but in the others there was no essential difference.

The response in the clinical manifestations of thyrotoxicosis was in keeping with the changes in basal metabolic rate. The thyroid gland generally remained the same size or became smaller.

No definite ill-effect from therapy was observed in any case. One individual while receiving therapy developed generalized aching, malaise and sore throat. The white blood cell count was found to be 2,600, with no change in the differential blood count. The white blood count returned to normal without interruption of therapy. In view of these findings we are inclined not to attribute the illness to a toxic action of the drug.

The response to from 100 to 200 mg. daily seemed to be as good as with 200 to 300 mg. The maintenance dose appeared to be between 50 and 100 mg. daily.

Five patients were subjected to thyroidectomy following preparation with iodothiouracil. In 4 the remission was not complete at the time of operation, 3 had had severe thyrotoxicosis, and coronary heart disease was present in another. However, the operative and postoperative course of each was uneventful. Less difficulty was encountered in removing the gland than is ordinarily the case after several weeks' preparation with the customary doses of thiouracil and Lugol's solution. There was not much bleeding or friability of the gland; there was only slightly more vascularity of the glands than in nontoxic goiters. Relatively little change in the pulse rate or blood pressure during the operation or in the pulse rate and temperature following it, was noted. In each of the 5 cases the thyroid gland was found to be well involuted histologically, as illustrated in Figures 3 and 4.

within four weeks after cessation of therapy the metabolic rate was plus 46 per cent. Thus, whereas 50 or 60 mg. daily failed to control the thyrotoxicosis, 90 mg. daily was successful.

Case 2. In Figure 6 is presented the response to therapy, as exemplified by the basal metabolic rate, of a housewife, aged 41, who had had manifestations of thyrotoxicosis for six months. The thyroid gland was enlarged diffusely to approximately 60 Gm. With 50 mg. of iodothiouracil twice daily for nine days the basal metabolic rate decreased from plus 25 per cent to 0. After 50 mg. daily, in one dose, for twelve days the rate was plus

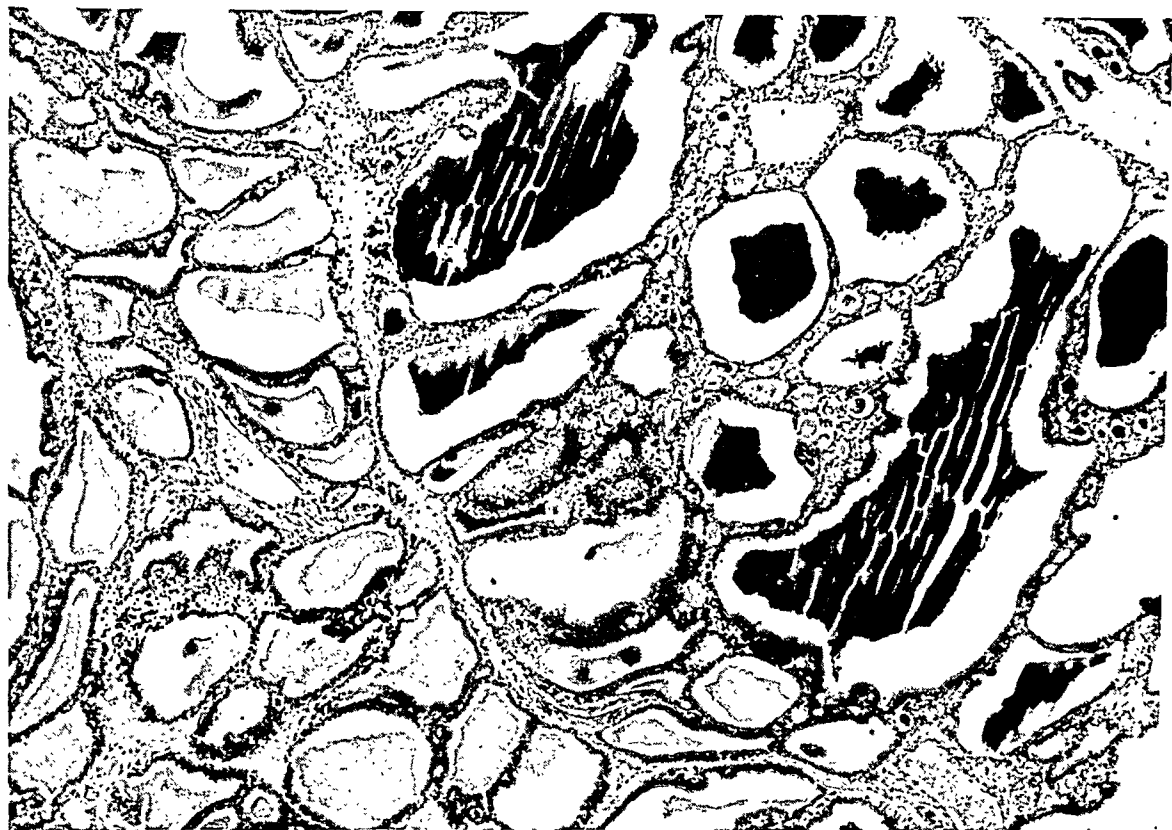


FIG. 3. Photomicrograph of thyroid ($\times 100$) of patient whose metabolic response to therapy is shown in Figure 8.

8 per cent. Therapy then was discontinued. Three months later the basal rate was plus 39 per cent; after 50 mg. twice daily for eight days it was minus 8. Therapy was again discontinued. Six weeks later the metabolic rate was plus 40 per cent, and after therapy for nine additional days it was plus 11 per cent. Therefore, this patient experienced three separate and complete remissions of thyrotoxicosis. Each of the first two remissions occurred very rapidly but the third one required a longer interval.

Case 3. In Figure 7 is illustrated the response to therapy of a fireman, aged 35, who had had thyrotoxicosis for three years. After 50 mg. of propylthiouracil three times daily for sixty days, the basal metabolic rate decreased from plus 89 per cent to plus 25; after 100 mg. three times daily for thirty days, the rate was plus 40. After an interval of eight months with no therapy the metabolic rate was plus 59 per cent. Then, following 50 mg.

TABLE 1—Continued

Patient No.	B. M. R. %			Maximal total daily dosage (mg.)
	Initial	Maximal response	Days required	
#130	+61	+23	18	200
#131	+14	+ 1	8	90
#134	+55	+21	7	300
#135	+18	+ 9	15	150
#137	+55	+12	43	150
#138	+13	+ 9	18	150
	+35	+18	21	150
#139	+83	+10	9	150
	+41	+36	9	300
#140	+70	+22	68	300
#141	+60	+38	7	250
#143	+34	+21	7	150
#145	+69	+21	33	200
#146	+29	+10	35	200
#147	+24	+ 9	15	150

Some of the responses to therapy can be illustrated by consideration of a few individual cases.

Case 1. In Figure 5 is presented the course of one patient as illustrated by change in the basal metabolic rate. The patient was a fireman, aged 38, who had manifestations of thyrotoxicosis for three months. The thyroid was diffusely enlarged, weighing approximately 30 Gm. The basal metabolic rate was plus 26 per cent. With 150 mg. of iodothiouracil daily for twelve days the metabolic rate decreased to plus 7 per cent and the manifestations of thyrotoxicosis disappeared. After 100 mg. daily, divided in two doses, for seven additional days the rate was plus 5 per cent. Then upon giving only 50 mg. daily in one dose for two weeks, the metabolic rate increased to plus 22 per cent; after 50 mg. twice daily for twelve days, the rate was plus 9; after 30 mg. thrice daily for two weeks it was plus 6, and it was maintained within a normal range for the next four weeks. However,

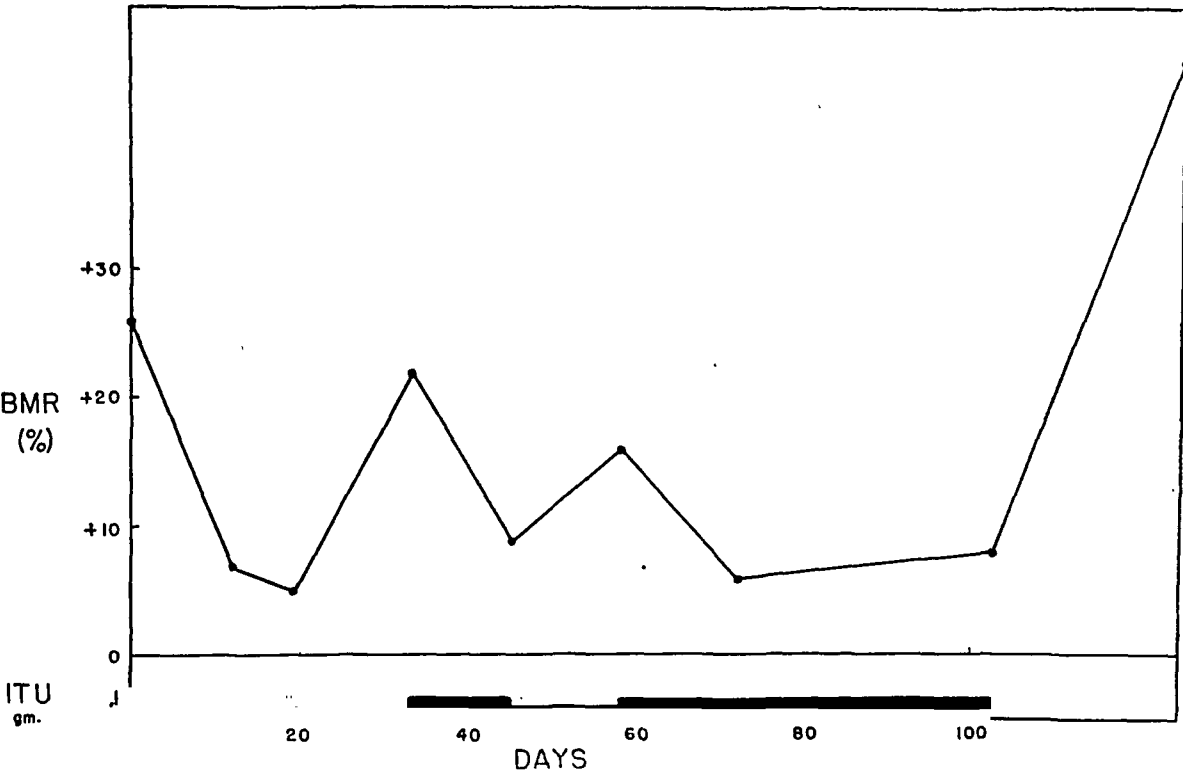


FIGURE 5.

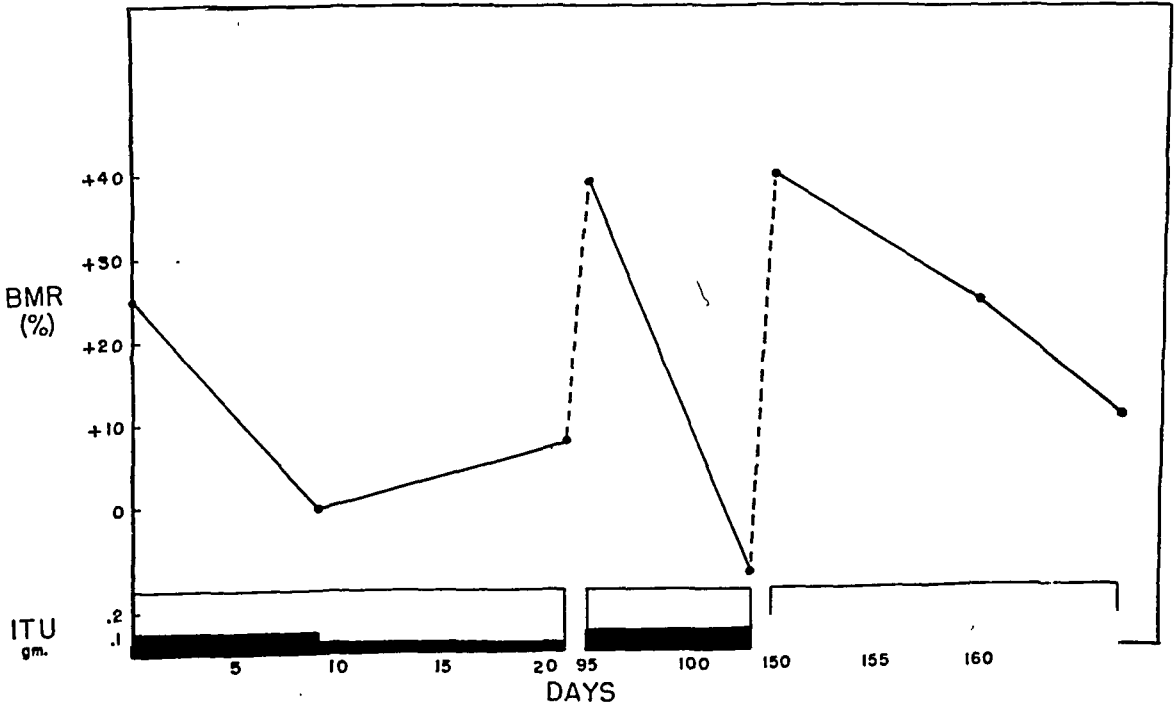


FIGURE 6.

of iodothiouracil three times daily for ten days, the rate was plus 3 per cent. The dosage was reduced to 50 mg. twice daily and after seven days the rate was plus 5. Therapy was then discontinued for five weeks. At the end of this interval the metabolic rate was plus 60. Propylthiouracil was given in doses of 100 mg. thrice daily and after nine days the metabolic rate was unchanged. Following this, iodothiouracil was given for five weeks, beginning with 30 mg. three times daily and gradually increasing the dosage to 50 mg. four times daily. The basal metabolic rate did not become normal again, but the patient experienced an almost complete remission of the thyrotoxicosis, clinically. The response was much slower than with the first course of therapy.

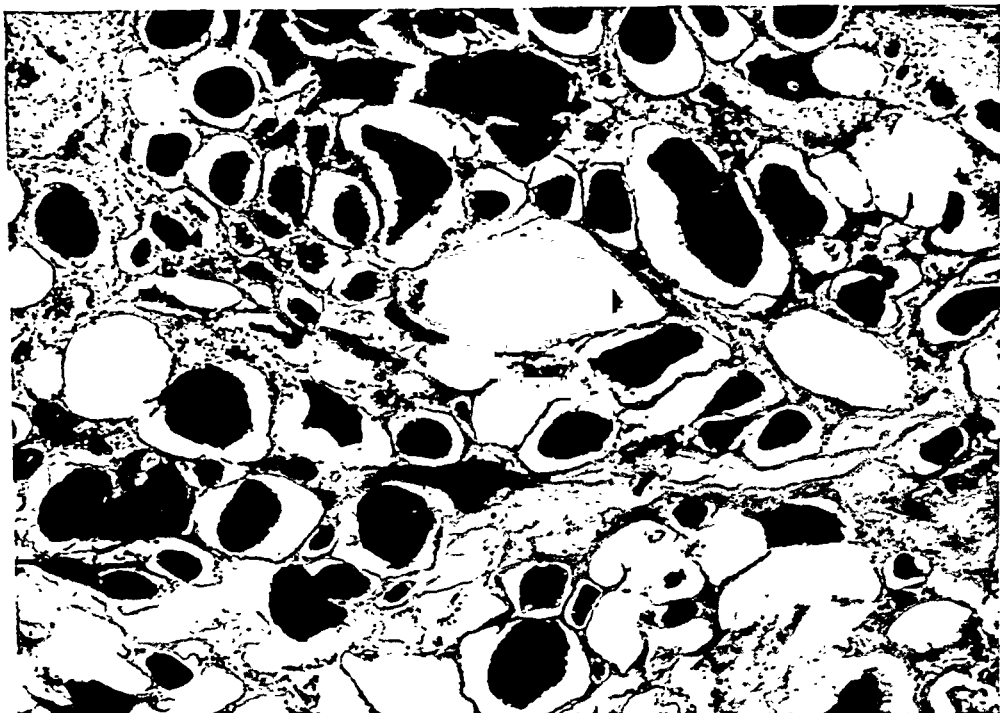


FIG. 4. Photomicrograph of thyroid ($\times 100$) of a patient, aged 71, with moderately severe thyrotoxicosis and enlargement of the thyroid to approximately 35 Gm. Iodothiouracil was given for forty-five days in total daily doses beginning with 150 mg. and gradually increased to 250 mg. The patient became euthyroid. A subtotal thyroidectomy was performed and 35 Gm. of tissue was removed. The operative and postoperative courses were smooth. Very little bleeding or friability of the gland was observed. Histologic examination revealed marked involution throughout.

Case 4. In Figure 8 is illustrated the course of a patient who was treated for seventy days with doses which were larger than those given to most of the other patients, but without experiencing a complete remission of his thyrotoxicosis. He was a laborer, aged 50, who had severe thyrotoxicosis and pituitarigenic ophthalmopathy. The thyroid was enlarged diffusely to approximately 45 Gm.; the basal metabolic rate was plus 70 per cent. After prolonged therapy with iodothiouracil, he experienced a distinct clinical improve-

to normal in 15 to 20 days." These investigators used as initial doses 600 mg. daily of thiouracil and 5 minims thrice daily of Lugol's solution. In certain of our cases 90 mg. of iodothiouracil daily was the maximum quantity used to produce a remission. In no instance was there good evidence that a daily dosage greater than 150 mg. was superior to the latter. It is to be emphasized that with 150 mg. of iodothiouracil only approximately

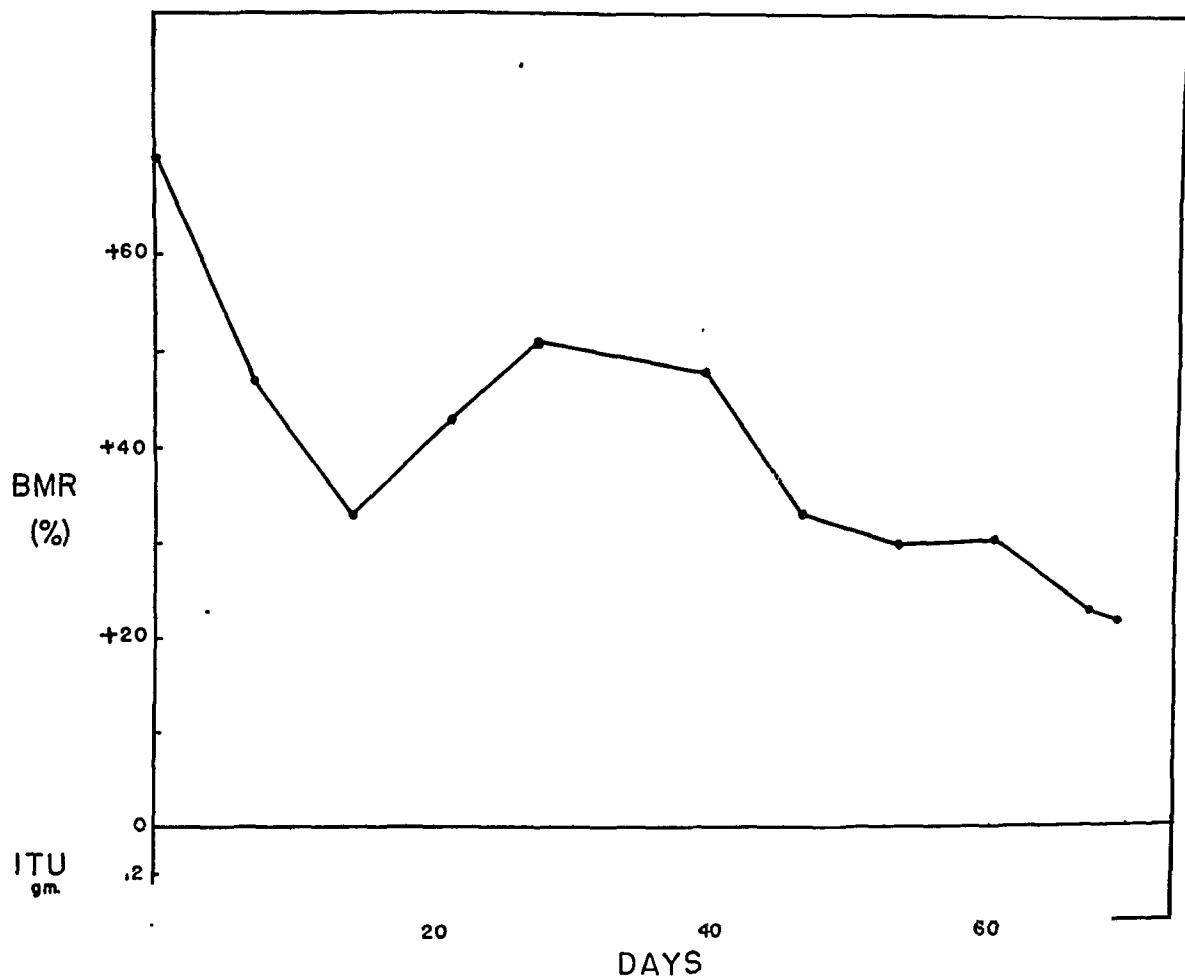


FIGURE 8.

75 mg. of thiouracil is being received. Within fifteen days this quantity of thiouracil has a negligible effect upon a patient with definite thyrotoxicosis. The quantity of iodine in 150 mg. of iodothiouracil is approximately 75 mg. which is the amount in two or three drops of a saturated solution of potassium iodide. Although much larger doses of iodine are usually given, the studies of Thompson (8) and Lerman (9) indicate that rarely is a dose in excess of 6 mg. necessary.

Whether iodide and thiouracil given in doses comparable to the respective amounts used in the iodothiouracil therapy are as effective as is the latter can only be determined by conducting this specific experiment.

ment, but he always remained thyrotoxic. The iodothiouracil was administered for seventy days and was the only antithyroid therapy which he received preoperatively. He had a smooth operative and postoperative course. His pulse never rose above 100 and there was very little bleeding or friability. The gland weighed 43 Gm. As shown by the photograph in Figure 3, the tissue showed relatively good involution.

The data that have been presented indicate that iodothiouracil can in some cases produce a rapid and complete remission of thyrotoxicosis. In Figure 9 comparisons are made of the maximal fall in basal metabolic rate

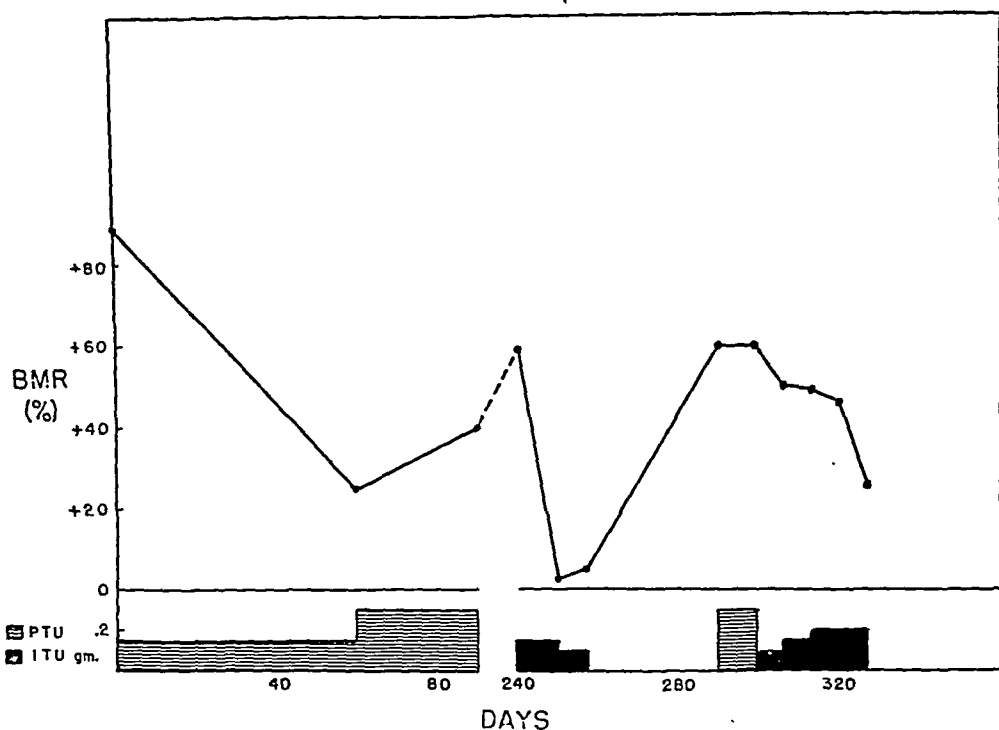


FIGURE 7.

in response to thiouracil, iodide and iodothiouracil. The maximal responses to iodothiouracil are not as good as the ones to thiouracil but better than the ones to iodine. The results of Maxwell, Gunter and Schwarz (7) using standard doses of thiouracil and iodide show essentially the same distribution as iodothiouracil when plotted in the same manner as the ones in Figure 9. However, this type of plotting does not take into consideration the dosage of the compounds used or the interval required to produce the response. Maxwell and associates state, "our experience is that thiouracil and iodine given concurrently from the commencement of treatment would in most cases reduce such a basal metabolic rate (plus 55 per cent)

produced occurred in the first seven to fifteen days of therapy, (d) the effects of 100 to 150 mg. daily were essentially as good as those from 200 to 300 mg., (e) exacerbations in thyrotoxicosis occurred upon changing from iodothiouracil to propylthiouracil, even though the same dosage of the latter had previously been effective, (f) the operative and postoperative responses to therapy, as well as the histologic appearance of the thyroid, simulated those of iodide therapy more than those of thiouracil, and (g) although certain patients had normal basal metabolic rates and were free of thyrotoxicosis, the disease was much more readily precipitated by emotional disturbances in them than in individuals in whom the same clinical response had followed thiouracil.

Although the results with iodothiouracil simulate qualitatively those obtained with iodide we have the impression, but not the proof, that it is somewhat more effective than iodide. Whether the thyroid concentrates iodothiouracil sufficiently to afford a more constant supply of iodine than is afforded when equivalent amounts of iodine are given as potassium iodide, is not known. Barrett (10) showed that rats fed amounts of iodide and thiouracil equivalent to the constituents of each in iodothiouracil, concentrated only approximately one-half as much iodine in the thyroid as when the latter compound was given alone. Since in each instance a subnormal amount of iodine was found, it is difficult to know whether there was less of a thiouracil effect or whether some of its action was counterbalanced by the iodine which it contributed.

5-Iodo-6-Methyl-2-Thiouracil

In view of the fact that methylthiouracil exerts a somewhat stronger antithyroid action than thiouracil, hope was entertained that the iodinated compound might also be more potent. However, quite the reverse was found in 6 patients with thyrotoxicosis (*see* Table 2). Three of these subjects had complete remissions with 5-iodothiouracil both before and after the methyl derivative was administered; a relapse was permitted to develop before each course of therapy was begun. A fourth subject (#123) had essentially a complete remission to 5-iodothiouracil before and after the course with the methyl compound. Patient #125 had a complete remission to iodothiouracil, but did not receive a second course of it. The response of patient #130 was essentially the same with the two compounds. In none of the 6 patients treated with iodomethylthiouracil was there better than a slight clinical response. One would think that some of the iodine would be liberated and that at least a common iodine response would be obtained. The difference in the effects of the two iodinated compounds is of great interest, but is not clear.

Our results indicate that the responses obtained with iodothiouracil are much more like those of iodide than of thiouracil. Among the similarities may be mentioned: (a) variations in response by different people, or even by the same individual who received several courses of therapy, (b) very rapid and complete response in certain individuals, (c) most of the changes

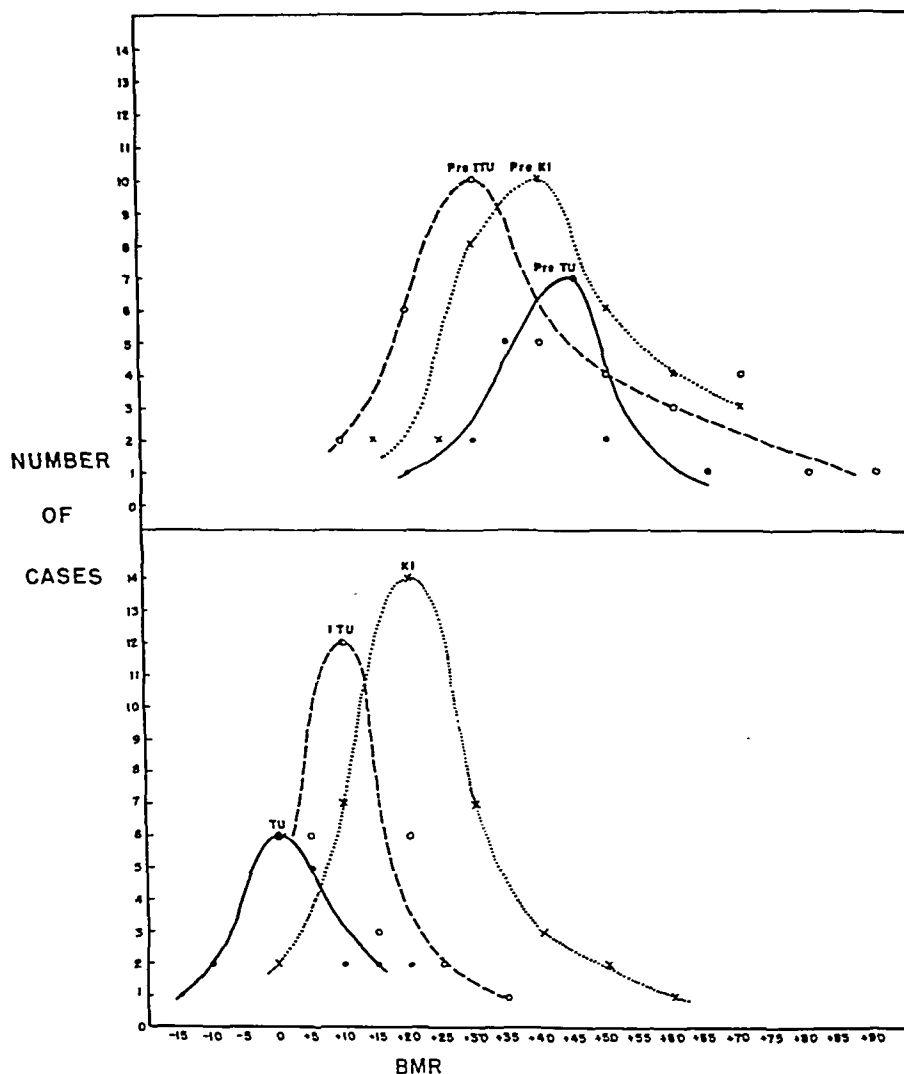


FIG. 9. In the top graph is shown the frequency distribution range of basal metabolic rates before therapy and in the bottom one is presented the response following iodine, thiouracil, or iodothiouracil. The data showing the responses to iodine and thiouracil were taken from Moore and collaborators (6). It is apparent that a greater proportion of patients had a fall in basal metabolic rate to a normal range following iodothiouracil than after potassium iodide.

viously. He found that 100 mg. showed no effect and, therefore, by this test its activity was less than 25 per cent of a comparable amount of thiouracil.

Thiocytosine

Dr. Astwood also tested this compound, in the manner previously described, and found that its activity was less than 25 per cent of that of thiouracil.

The goitrogenic effect of thiocytosine in rats was found to be similar to that of thiouracil (Table 3).

One patient with severe thyrotoxicosis was given 50 mg. of thiocytosine three times daily for twenty-one days. Although the basal metabolic rate decreased from plus 60 to plus 41 per cent there was no definite clinical improvement. No additional supply of the compound could be obtained for testing other patients.

SUMMARY AND CONCLUSIONS

Forty courses of therapy with 5-iodo-2-thiouracil were administered to 29 patients with thyrotoxicosis. The responses simulated qualitatively those following iodide therapy more than those which thiouracil produces. In 15 patients the maximal response occurred within ten days and in 21, within fifteen days. The individuals who had not become euthyroid within fifteen days did not attain this state with further continuation of such therapy. Five patients who received iodothiouracil in preparation for subtotal thyroidectomy experienced a smooth operative and postoperative course. The thyroid gland revealed a relatively good degree of involution.

Iodothiouracil inhibited the uptake of radioiodine by the thyroid of rats slightly more than did an equimolar dose of thiouracil, but less than an equivalent amount of iodine given as potassium iodide.

5-Iodo-6-methyl-2-thiouracil was used in the treatment of 6 thyrotoxic patients. It was much less effective than was its nonmethylated analogue.

(Ca)-4-n-propyl-6-oxypyrimidyl-2-mercaptoacetic acid was used in the treatment of 5 patients with thyrotoxicosis and thiocytosine was employed in 1 subject. No patient received significant benefit from either drug. Each compound was distinctly less active than was thiouracil in antagonizing the uptake of radioiodine in the thyroid by normal subjects who had received tracer doses of the isotopes several hours previously.

Thiocytosine exhibited essentially the same amount of goitrogenesis in rats as did thiouracil.

None of the patients experienced a toxic reaction to any of the compounds studied.

TABLE 2

Compound tested	Patient No.	B. M. R. %			Total daily dosage (mg.)
		Initial	Maximal response	Days required	
5-Iodo-6-methyl-2-thiouracil	#112	+26	+37	9	100
	#115	+26	+32	19	150
	#123	+45	+27	25	150
	#125	+37	+63	36	150
	#129	+49	+54	15	100
	#130	+40	+18	28	150
(Ca)-4-n-propyl-6-oxypyrimidyl-2-mercaptoacetic acid	#125	+32	+48	47	200-400
	#127	+17	+21	28	250-400
	#135	+27	+24	8	250
	#141	+37	+60	21	250-400
	#151	+45	+29	35	300-400
Thiocytosine	#139	+60	+41	21	150

(Ca) 4-n-Propyl-6-Oxypyrimidyl-2-Mercaptoacetic Acid

This compound was used in the treatment of 5 patients, in total daily doses ranging from 200 to 400 mg. Little improvement was observed in any patient, although one was treated continuously for forty-seven days and another for thirty-five days. No toxic reactions were encountered.

Dr. Astwood kindly tested the effect of this compound upon the concentration of radioiodine in the thyroid, in the manner discussed pre-

TABLE 3

Amount of compound			Thyroid size	
Per cent solution	Mg. consumed per 100 Gm. body weight		Mg. per 100 Gm. body weight	
Compound	Thiouracil	Thiocytosine	Thiouracil	Thiocytosine
.0001	0.3	0.4	7.8	8.6
.0005	1.6	1.5	8.1	8.2
.001	2.4	3.2	6.7	7.7
.005	14.4	13	8.7	8.3
.01	23.8	30	11.4	9.5
.1	200	244	17.2	17.9

RETARDED ABSORPTION OF PELLETS OF PROTAMINE-ZINC INSULIN

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AFTER Parkes and Young (1939(1)), Mark and Biskind (1940(2)), Cutting, Morton and Cohn (1941(3)) and Parkes (1942(4)) proved the inefficacy of subcutaneous implants of insulin, either pure or with cholesterol, this experimental path was apparently abandoned. McCullagh and Lewis (1942(5)) reported prolonged effects with subcutaneous implantations of tablets of insulin-cholesterol in 4 dogs and of silver tubes filled with crystalline insulin and open at one end, in 5 others. All dogs were normal. A definite decrease in the blood sugar was evident for a period of twenty-one days. Potent insulin was found in the removed tissue-encapsulated remnants of the implants. In spite of these discouraging results, we decided in 1943 to carry on the study of insulin implants, a therapeutic method which was giving excellent results with liposoluble hormones in clinical medicine. Our experiments in normal (Vargas, Koref and Zañartu, 1944(6)) and diabetic rabbits (Vargas, Koref and Lewin, 1948(7)) implanted with a protamine-zinc-insulin complex resulted so satisfactorily that one of us has been able to apply it in clinical practice (Vargas and Lewin, 1948(8) and Vargas, 1949(9)).

METHODS

All the experiments were carried out in normal rabbits and in rabbits made diabetic by alloxan (Dunn *et al.* 1943(10)) or alloxantin (Koref *et al.* 1944(11)). Males and females were used indifferently, their weight varying between 1,000 and 2,000 Gm. Their food was fresh grass, carrots, compressed rabbit's pellets¹ and water *ad libitum*. We used crystallized insulin from the Instituto Bacteriológico de Chile, and amorphous insulin from the Instituto Sanitas in order to make the protamine-zinc-insulin in a way similar to the commercial methods. The tablets or pellets of insulin, with or without cholesterol, were prepared directly from the crystalline or amorphous powder. Those of protamine-zinc-insulin were made from the powder, in the following way: the PZI complex obtained at pH 7.1, as in the commercial method, was filtered through a hard filter paper; the pre-

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¹ Manufactured by Williamson, Balfour & Co. and containing wheat, alfalfa, peanuts, NaCl and CaCO₃.

The results with 5-iodothiouracil are sufficiently encouraging to warrant further testing of this compound and other similar iodinated ones in the preparation of thyrotoxic patients for thyroidectomy. Comparisons of the effects of equimolar quantities of iodide and thiouracil should also be made.

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varied between 488 and 778 international units, did not cause death before the sixth day. However, the animals finally died in hypoglycemic shock. With all the other forms of insulin used, the majority of the nondiabetic animals survived with no change in their normal growth. The glycemia curves showed a depression during fasting, which in the cases treated with PZI or PZI-cholesterol lasted for approximately fifty days after the implantation (Fig. 1).

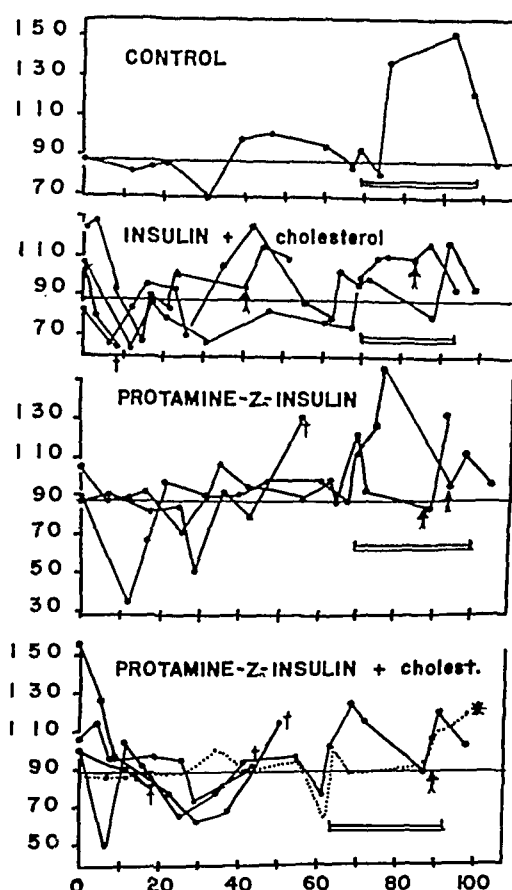


FIG. 1. Values for blood sugar, after a 12-hour fast, in normal rabbits implanted subcutaneously with tablets of different types of insulin.

Abscissae, time in days; ordinates, milligrams of glucose per 100 cc. of blood. The double horizontal lines in the 70–90 day area indicate that values were obtained without fasting. The implantation was made on 0 day. Arrows indicate extraction of the tablet; † spontaneous death; * the tablet was not found.

It was observed that the tablets of PZI (after 5, 91 and 96 days) and of PZI-cholesterol (after 22 and 100 days) still contained insulin, producing a lowering of blood sugar when an extract of them was injected into normal rabbits. This effect was absent in recovered pellets of insulin-cholesterol. The tablets of insulin-cholesterol have almost the same weight before and after the implantation, even increasing their weight in some

cipitate, together with the filter paper, was placed in a vacuum desiccator containing sulphuric acid and when dry, it was gathered from the filter paper and powdered in a small mortar. When the mixture with cholesterol (c.p. Pfanstiehl) was used, the proportion was 1:1.

The tablets were made in a small tableting machine. Their diameter was 6 mm. and their weight varied between 22.2 mg. and 58.6 mg. The pellets were made in a special apparatus which insured uniform compression; their diameter was 1.5 mm., their length varied between 2 and 10 mm. and they weighed between 3 and 14 mg.

The material was implanted in the subcutaneous tissue and in a few instances, in the muscle. The glycemia was determined by the Hagedorn-Jensen micromethod, usually after a period of twelve hours' fasting. The tablets and pellets, when removed from the animals, were freed from the capsule, dried and weighed. In 63 normal animals, 88 implantations were performed; and in 11 diabetic rabbits, 17 implantations. Table 1 shows

TABLE 1. DETAILS OF THE IMPLANTATIONS PERFORMED IN
NORMAL AND DIABETIC RABBITS

Group	No. of animals	Number of implantations				Form
		Insulin	Insulin-chol.	PZI	PZI-chol.	
Normal	63	10	<i>Subcutaneous</i>		15	Tablets Pellets
			4	10	35	
		—	<i>Intramuscular</i>		3	Tablets Pellets
			—	—	11	
Diabetic	11	—	<i>Subcutaneous</i>		7	Pellets
			4	1		
		—	<i>Intramuscular</i>		5	Pellets
			—	—		

the details. Among the 63 implanted normal animals, glycemia was tested for in 12. In the diabetic animals, in addition to determining the amount of glycemia, tests were also made for glycosuria (Fehling's method) and acetoneuria (Gerhardt's method). The body weight was determined in both groups two or three times a week.

RESULTS

Insulin alone, implanted in tablet form into normal rabbits did not avoid rapid absorption, even though it was observed that the implants, which

TABLE 2. IMPLANTATIONS MADE IN ALLOXAN-DIABETIC RABBITS

Animal No.	Sex	Weight		Alloxan 5% mg./Kg.	Implantation				Days of Observation			General	Average blood sugar		W. varied the imp.
					Form	Mg.	IU	IU/Kg.	Before implant	During implant**	Total		Pre-implant	During implant	
32/38	F	2700	2700	200	Implantation of PZI				11	14	25	insufficient		390† (382-400)	—
32/36	M	1520	2350	100	Implantation of insulin and cholesterol (40%)				24	8	91	insufficient	285 (273-298)	—	—
					T. Subcutan.	29.4	353	200				insufficient		275 (181-343)	—
					T. Subcutan.	58.5	702	360		27		insufficient		247 (80-328)	—
					T. Subcutan.	108	1296	550		28		insufficient		40	—
40/57	F	950	1340	200	T. Subcutan.	91	1092	870	5	2	7	excessive	337		—
1/14	F	2420	1920	135	Implantation of PZI and cholesterol (50%)				22	43	78	insufficient	384 (351-400)	270 (204-349)	—
32/15	M	2520	1800	198	T. Subcutan.	27.8	278	130		4		insufficient		—	—
					T. Subcutan.	33.4	334	160		9		insufficient		—	—
					T. Subcutan.	56.2	562	280		3	11	insufficient	390	385	—
32/91	M	1800	2200	200	T. Subcutan.	29.8	298	140	7	1		insufficient		350	—
					T. Subcutan.	68.4	684	350	10	61	71	insufficient	228	166 (121-193)	—
36/7	F	1600	1460	187	T. Subcutan.	28.8	288	140	7	7	14	excessive	242	26	—
36/22	F	1950	1700	200	P. Intramusc.	67.1	671	510	8	80	88	good	252	121 (88-235)	—
36/34	F	2300	2620	185†	P. Intramusc.	100.0	1000	600	7	22	84	good	202	60 (33-77)	—
1/50	F	2300	2620	43	P. Intramusc.	149.2	1492	690		48		good			—
					P. Intramusc.	153.0	1530	680				good	316 (313-320)	263 (196-326)	237 (166-304)
1/30	M	2300	1825	54 41 200	P. Subcutan.	153.0	1531	430	14	227	801	good		158 (70-245)	96 (70-140)
					P. Intramusc.	199.0	1990	820#	18	122	153	insufficient	338 (317-374)	356 (334-400)	—

* T = Tablets. P = Pellets.

** Period during which the implant remained in the body.

† The glycemia was determined five days after the implantation. The figures in parentheses correspond to minimum and maximum results.

‡ Alloxantin 2.5%.

Many pellets (about 50%) eliminated after the nineteenth day of the implant.

cases. This fact prevented calculation of the rate of insulin-cholesterol implants.

If in normal rabbits there is a prolonged insulin alloxan diabetes it is even more evident. The animals in this series as a control lived between four and six days. The diabetic animals treated with a sufficient dose of PZI-cholesterol, showed a tendency towards complete recovery. It was possible to observe them for over two hundred days. In the diabetic animals the best results were obtained with implants of 690 units per kilogram of body weight. Only animal No. 36/7 died from hypoglycemic shock, six days after the implantation of 510 units per kilogram of PZI-cholesterol. However, this animal was in a bad nutritional condition after the administration of alloxan, having lost 290 grams in seven days, and the shock occurred after a fasting period of eighteen hours. Of especial interest is animal No. 1/50 which, after presenting a very severe diabetes, received 2 implants of PZI-cholesterol (1,530 and 1,331 units, respectively) and recovered completely from its diabetes. Biopsy specimens taken from the pancreas 170 days after the first implantation and later, 861 days after the injection of alloxan and 365 days after the second implant, showed an evident damage of the beta cells of the islands of Langerhans in the first histologic specimen, but signs of regeneration and frank cellular recuperation in the second specimen (Gomori's stain). Figure 2 shows the evolution of the diabetes in animal No. 1/50. Figure 3, as an example of insufficient treatment, shows the evolution of the disease in animal 1/30, in which on the nineteenth day the implant was partially eliminated due to suppuration. Animal No. 1/30 is a control for animal No. 1/50. Both had a similar degree of diabetes, both received similar treatment with alloxan and PZI implants, at the same time. During the pre-implant period (thirteen days) both required about five injections of PZI (daily dose 10 to 16 I.U.). The high requirement of insulin of No. 1/30 is again demonstrated when, on the thirty-fifth day after the implantation, insulin injections were needed again. From then on, twenty-three injections were given in increasing doses, the last being 80 I.U., without control of the diabetic condition.

Absorption

The calculation of the absorption of the insulin was based on the difference in weight of the pellets before the implantation and after their recovery. The method was similar to that used by other writers for mixtures of liposoluble hormones with cholesterol.

The implants were removed after a certain number of days; if one animal received several implants at a time, these were removed at different

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time
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times. The difference between the initial weight and that of the pellet (or tablet) taken from the animal and dried, was expressed in per cent of the original weight of the pellet (percentage of reabsorption). From the data on the percentage of reabsorption, and on the days during which the pellet remained under the skin of the animal, a graph was constructed.

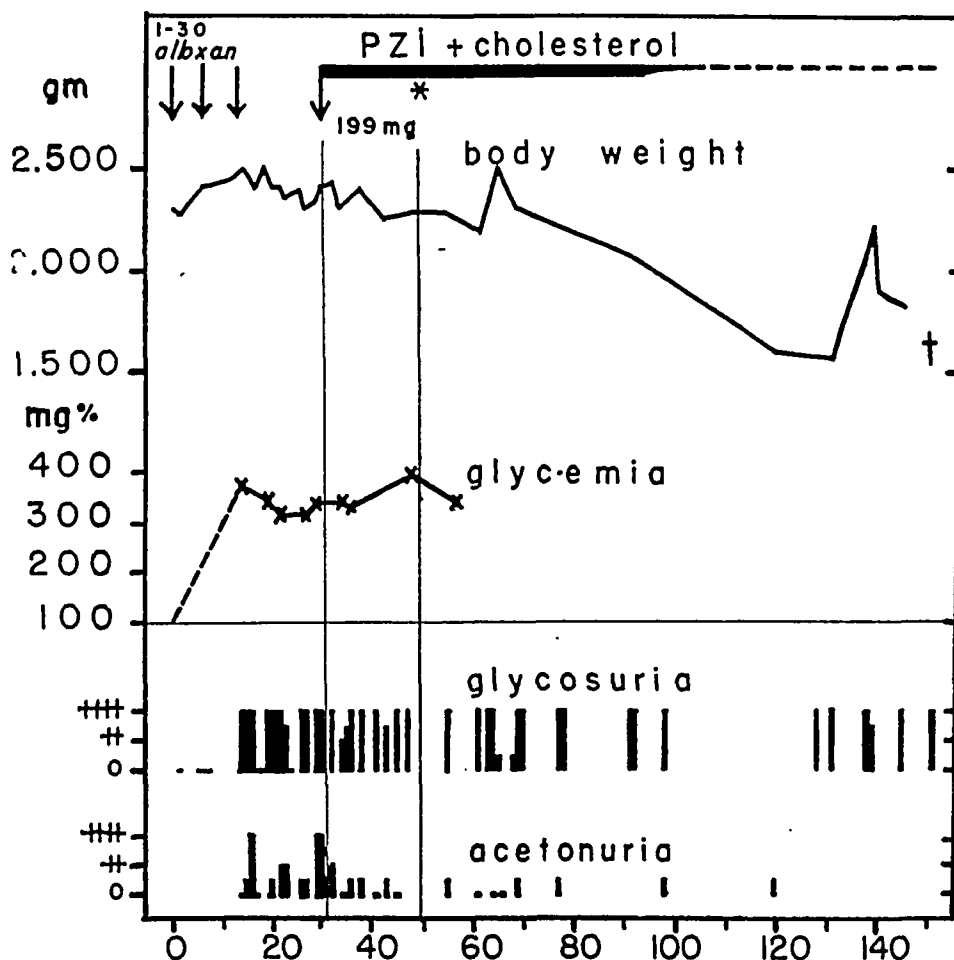


FIG. 3. Rabbit No. 1/30, with alloxanic diabetes treated insufficiently with only one implantation of PZI-cholesterol. The dose was divided into two parts, implanted into separate legs. The material was partly eliminated by abscess formation on one side.

Abscissae, ordinates and other abbreviations are the same as in Figure 2. Asterisk indicates expulsion of pellet.

The accompanying graph (Fig. 4) shows the absorption curve of the PZI-cholesterol implants. In relation to the percentage of absorption, the shape of the pellet does not seem to have any influence upon the rapidity of absorption of the substance. It is of interest to remember that we are working with a protamine-insulin complex, of which solubility in water is fairly low and of which the reabsorption is even more retarded by the reaction of the tissues (pH 7.0-7.1). The cholesterol only facilitates the

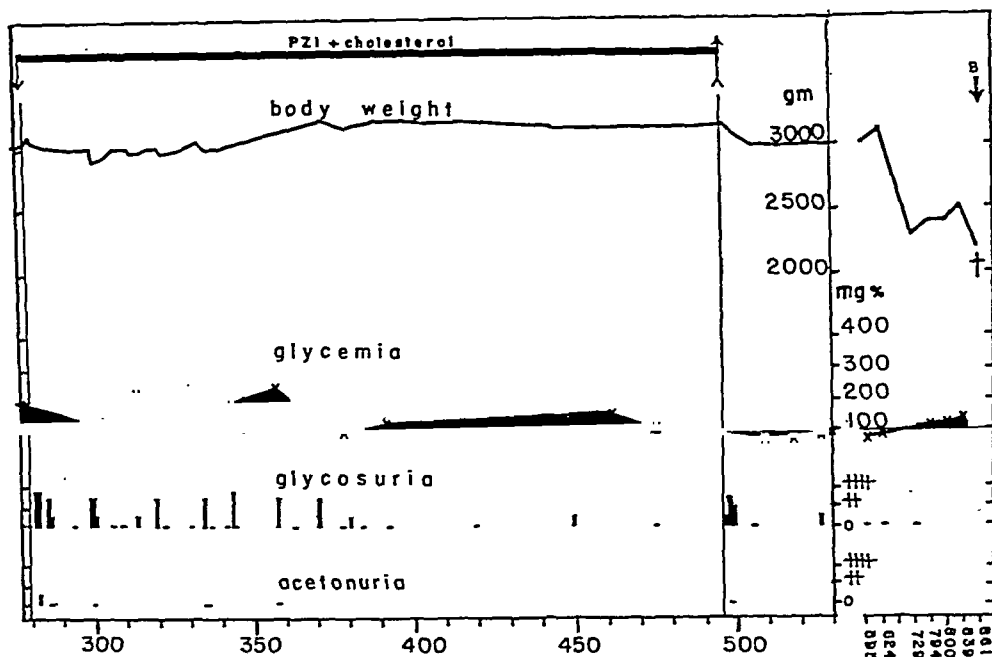
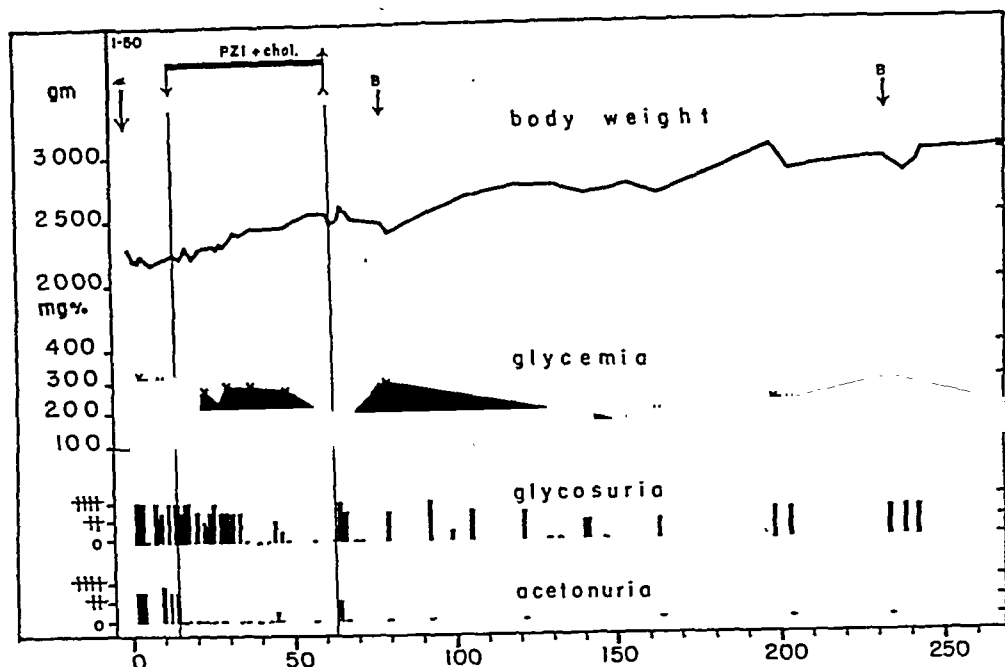


FIG. 2. Rabbit No. 1/50, with alloxanic diabetes treated with two implants of PZI-cholesterol. Above: first implant. Below: second implant. During the first pre-implant period of fourteen days, the animal received 7 subcutaneous injections of 10 to 20 I.U. of PZI (Lilly); after the first implant, these injections were suspended. Note that glycemia decreased and glycosuria-acetonuria disappeared during the period of the first implantation; complete normalization was attained during the second implantation and persisted almost indefinitely.

Abseissae, time in days; arrow "a," alloxan injection; arrow "B," biopsies of pancreas; arrows joined by horizontal line denote periods of implantation (the implant was removed at the end of the line).

previously the complex at the required pH. Their mixture made into pellets is absorbed as rapidly as insulin alone, being freely soluble in the tissue fluids. That is why pellets of this mixture in larger doses (261 and 1,012 units) produced hypoglycemic shock and in smaller doses (43–88 units) produced only a transitory lowering of the blood sugar, which lasted 48–100 hours. Furthermore, they stated that pellets prepared from the dry powdered commercial PZI complex, as described here by us, were inactive. They could not explain why the complex was altered by desiccation, in spite of the fact that the redissolution of one of these pellets, prepared from the commercial product, caused a lowering of the glycemia when injected. This shows that actually there was no alteration of the PZI and that the dry powder conserved its activity. The implants produced no change in the glycemia because the doses used were too small. When a few milligrams are implanted,¹ the absorption of the complex is so slow, (as we have already shown), that it is not enough to influence the glycemia either in the normal or in the diabetic animal. It is the same as if an insufficient dose of insulin was injected. In this way Mark and Biskind failed to ascertain the useful dose.

Cutting *et al.* (3), implanted pancreatectomized dogs with pellets of insulin-cholesterol and obtained an effect upon the glycemia which lasted as long as thirteen days. These results agree with ours, since we have shown that cholesterol prolongs the absorption of pure insulin although it is not capable of avoiding a relatively fast absorption.²

SUMMARY

1. It has been shown experimentally in normal rabbits that protamine-zinc-insulin is the only type of insulin useful for subcutaneous implantations. This protamine-zinc-insulin compound, either in tablets or in pellets, alone or mixed with 50 per cent cholesterol, is absorbed so slowly that its lowering effect on the blood sugar lasts at least as long as fifty days.

2. Subcutaneous or intramuscular implants of protamine-zinc-insulin complex with 50 per cent of cholesterol in alloxan diabetic rabbits, when used in doses of from 430 to 690 units per kilogram of body weight, control the diabetes and metabolic changes for approximately one hundred days.

3. The results with this type of implant enable us to calculate a daily absorption of approximately 1 per cent of the amount implanted. This

¹ The authors do not state the dose used in their experiments but it must have been the same as that used with the other material they implanted, *i.e.*, 4.0 mg.

² We take this opportunity to thank Drs. Jorge Lewin and Juan Villalobos for their help in the preparation of the PZI-cholesterol pellets and in controlling some of the implants.

compression and permits a better conservation of the shape of the pellet. Although we have not studied the curve of absorption of PZI without cholesterol, the few points shown in the graphs indicate the same rate of daily absorption as for the PZI-cholesterol. This permits us to deduce that with this mixture the absorptions of insulin and cholesterol are probably proportional. The absorption is completed about the one hundred and thirtieth day. In general, we can state that the implant is conserved in the body for at least one hundred days, its absorption being slightly more

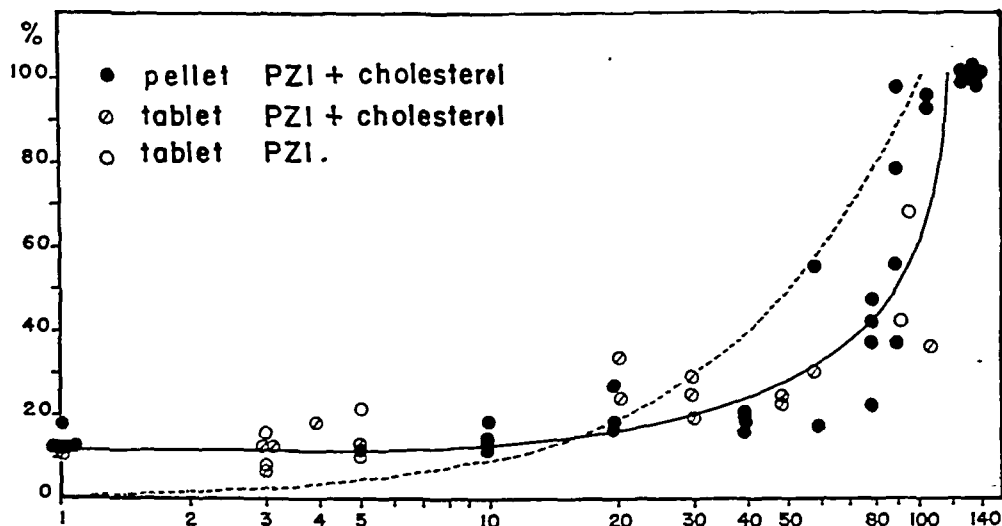


FIG. 4. Curve of absorption of tablets and pellets of protamine-zinc-insulin and PZI-cholesterol (50 per cent) in normal rabbits.

Abscissae, time in days in logarithmic scale; ordinates, total percentage of absorption. The dotted curve corresponds to 1 per cent daily absorption.

rapid in the last twenty or thirty days. This is probably due to the disintegration of the implant which gives it a greater surface of absorption. A daily absorption of approximately 1 per cent of the total quantity implanted is a fairly accurate practical calculation. Therefore, knowing the daily insulin requirement, it is easy to calculate the amount of insulin which should be implanted (*i.e.*, 20 units daily calls for an implant of 2,000 units).

DISCUSSION

Our results are contrary to those of Mark and Biskind (2). They stated that pellets of protamine plus zinc-insulin, in a proportion of 4:1, influenced the diabetes of pancreatectomized dogs for not more than 48–100 hours. However, their preparation of this mixture was different from ours because they simply mixed the protamine with the zinc-insulin without obtaining

THE BEHAVIOR OF LABELED IODOCASEIN IN HUMAN MYXEDEMA*

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STUDIES of the distribution and transformation of calorigenic, iodine-containing substances have been handicapped by the fact that pharmacologic doses of material were usually required in order to obtain a concentration of iodine in tissue or body fluids sufficiently high to be measured satisfactorily by current chemical methods. The availability of radioiodine (I^{131}) has made it possible to reinvestigate the metabolism of calorigenic iodine compounds in animals and man under physiologic conditions. Radioiodine can be incorporated into calorigenic materials so as to obtain biologically active compounds with high specific activity. This not only permits the use of physiologic amounts of material, but also permits determination of the distribution of the material and its derivatives with greater accuracy and sensitivity than was previously possible. In addition, radioiodine and substances containing it can, to some extent at least, be observed in vivo by means of a Geiger counter directed at various parts of the body.

The present study represents an initial effort to explore the distribution and transformation of calorigenic compounds labeled with radioiodine. Iodocasein, an artificial iodoprotein, was chosen for this study because it was felt that its synthesis from casein and radioactive iodine might be accomplished with less difficulty and less radiation hazard than that of other materials. Iodocasein has been proved to be actively calorigenic in animals and man (1, 2) and essentially similar in its biologic behavior to other iodoproteins which have previously been employed in man (3, 4).

A myxedematous patient having little or no thyroid function consented to be the subject of the study. This patient was chosen in order to minimize or avoid the possible complications which might result from the turn-

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estimate is appropriate for clinical application, and we recommend it for further study in human diabetics.

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After the initial radioiodine studies during myxedema were completed, a daily dose of 60 mg. of nonradioactive iodocasein was administered. Fourteen days after the initiation of this therapy, subjective and objective evidence of improvement was obvious. After thirty-seven days of treatment, the signs and symptoms of myxedema were completely alleviated. The basal metabolic rate had risen from -29 per cent to 0, the cholesterol had declined to 167 mg. per 100 cc. of serum, and the weight from 77.05 Kg.

TABLE 1. CHEMICAL AND ISOTOPIC CHARACTERIZATION OF THE MATERIALS ADMINISTERED DURING THE INVESTIGATION

Date given	Preparation no.	Material	Dose, mg.	Iodine content, mg.	I ¹³¹ content, micro-curies
9-18-47	1	NaI* + carrier	0.1	0.085	100
9-27-47	2	NaI* + carrier	3.62	3.0	175
10- 3-47	3	Radioactive iodocasein (P _M)†	70.0	3.5	170
10- 9-47 to 11-16-47	4	Nonradioactive iodocasein (P _C)†	60.0	3.7	
11- 4-47	5	NaI* + carrier	3.62	3.0	155
11-11-47	6	Radioactive iodocasein (P _N)†	63.0	3.4	230

* Radioactive.

† The abbreviations P_M, P_C and P_N refer to specific preparations described in detail elsewhere (8).

to 70.93 Kg. (Fig. 1). At this point, the patient was judged to be in a euthyroid state, and the studies with inorganic radioiodide and radioiodocasein were repeated. At the end of the study, which required fifty-seven days for completion, the patient was dismissed with instructions to take 1½ grains (0.1 Gm.) of desiccated thyroid¹ daily. During a year of observation which followed, she has remained euthyroid and in good health on this dose.

¹ The desiccated thyroid employed was Parke, Davis & Company strong uncoated thyroid, which is assayed by the manufacturer as 50 per cent stronger than U.S.P. thyroid.

over of labeled iodide (or perhaps of other catabolic by-products of the material given) by the thyroid gland. Considering the remarkable capacity of the thyroid to accumulate iodine and to synthesize it into thyroid hormone, it was anticipated that the presence of the thyroid gland might obscure the primary metabolic events following the administration of exogenous thyroid-like material which we wished to study. Once the extrathyroidal behavior of such materials has been evaluated, the effect of the intact thyroid upon them may well become a subject for future investigation.

MATERIALS AND METHODS

The plan of the study was to obtain several observations on the distribution and fate of labeled iodocasein in a patient with myxedema at two metabolic levels: 1) while hypothyroid, before treatment, and 2) while euthyroid, after adequate treatment of the myxedema with nonradioactive iodocasein. As control data, exactly similar observations were made with labeled sodium iodide in amounts comparable to that of the iodine contained in the iodocasein. Both labeled materials were administered by mouth.

1. Report of case. The patient selected was a married, white woman, aged 45 years, who had postoperative, postirradiation myxedema. In April, 1947, partial thyroidectomy with resection of the left lobe had been performed for nodular goiter without hyperthyroidism. The pathologic examination revealed a solid adenocarcinoma, grade 2 (Broders' method) with the extra-adenomatous portion of the thyroid showing marked thyroiditis and regenerative hyperplasia. After thyroidectomy, she had been given a course of radium therapy. The patient returned on September 9, 1947, with the signs and symptoms of myxedema, including characteristic changes of voice, facies and skin as well as slowed tendon reflexes. The body weight was 77.05 Kg. The basal metabolic rates were -26 and -29 per cent, and the concentration of cholesterol was 420 mg. per 100 cc. of serum. A tracer dose (for diagnostic purposes) of 100 microcuries of I^{131} as sodium iodide with 100 micrograms of stable sodium iodide as carrier revealed a total excretion in the urine of 86.9 per cent in seventy-two hours. This high urinary excretion is typical of myxedema (5).

The patient was transferred to the metabolism unit and placed on a 1,400 calorie repetitive diet, which contained a low but constant amount of iodine. The iodine content was not determined directly, however. The preparations used in this investigation and the order of their administration are given in Table 1. Clinically the patient was followed by daily observations of body weight, basal pulse and blood pressure, and determination at least twice weekly of basal metabolic rate and serum cholesterol.

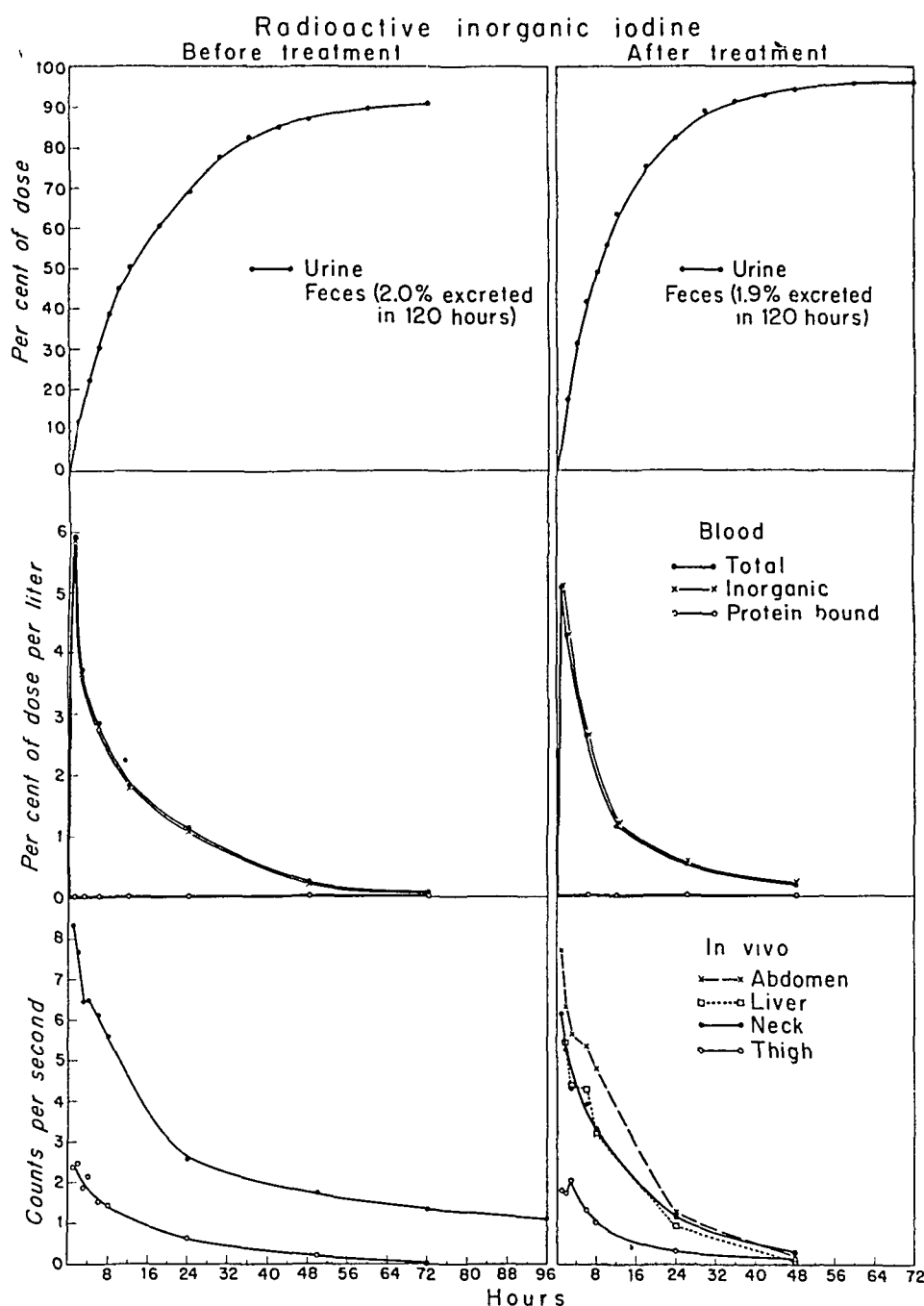


FIG. 2. The behavior of orally administered labeled iodide during myxedema and euthyroidism as indicated by measurement of urinary and fecal excretion, blood levels, and in vivo observations.

period, and if only small amounts of radioactivity were present, the stools were pooled for analyses. The Kendall (10) open ashing method of preparing the stools was used. In place of titration, however, 0.5 cc. of the final mixture was counted in wet form with a beta ray (thin mica window) counter. Determinations of radioactivity in the feces were checked by measurements with a gamma ray counter. Measurements of radioactivity

2. Laboratory methods. A) *Radioactive compounds*.—The preparation of labeled iodide for clinical use has been previously described (6). In the present study, enough NaI^{127} was added in order for the iodine content of the labeled iodide doses to equal the iodine content of the dose of labeled iodocasein, which was approximately 3.0 mg. (Table 1).

Iodocasein and radioiodocasein were prepared by a modification of the method of Reineke and Turner (7). The preparations used contained between 5 and 6 per cent of iodine by weight; 25 to 35 per cent of the iodine

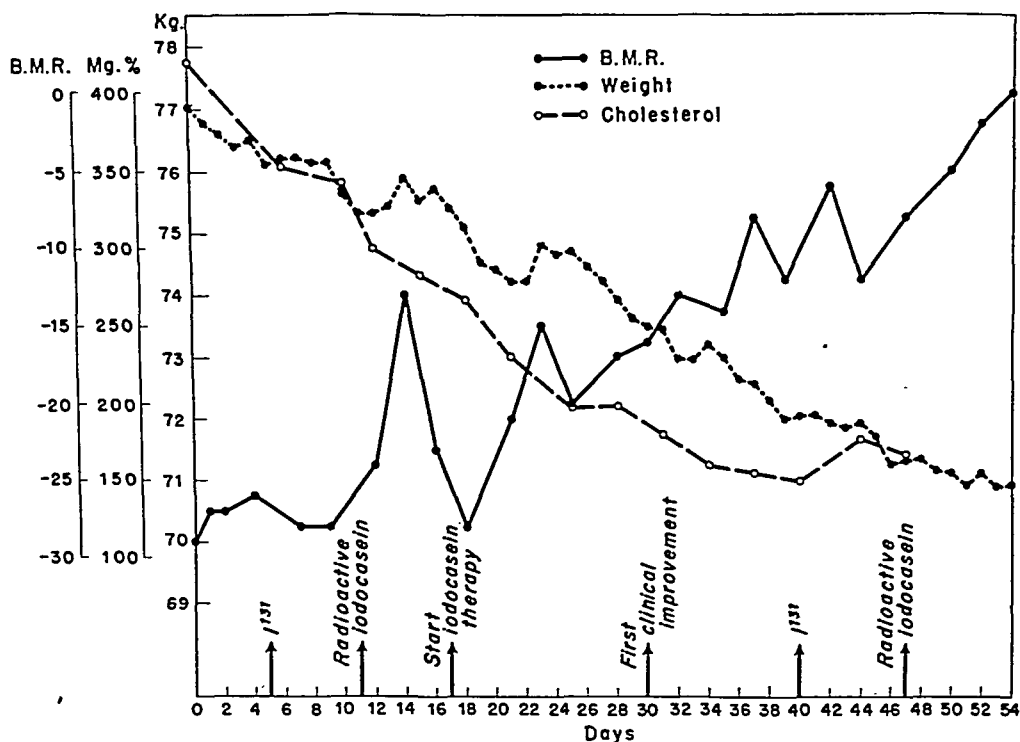


FIG. 1. The clinical course of the patient during therapy with iodocasein as indicated by changes in basal metabolism, body weight, and serum cholesterol.

present was combined as thyroxine. The technical procedures involved in preparation of iodocasein and radioiodocasein are described in a separate paper, in which characteristics and analyses of the preparations used in this study are described in detail (8). The biologic potency of the iodocasein preparations as determined by bio-assay in tadpoles of *Xenopus laevis* was compared with that of desiccated thyroid and a physiologic daily dose for the artificial hormone was estimated to be 60 mg. (9).

B) *Analyses of radioactivity*.—Urine was collected and analyzed as described elsewhere (6). Stools were collected separately over a five-day

tion of the labeled iodide. After forty-eight hours, however, when the total radioactivity was very small, I^{131} appeared in the precipitable fraction, which constituted more than half of the total serum I^{131} . When labeled inorganic radioiodide was ingested in the euthyroid state, all of the serum radioactivity remained in nonprecipitable form. The chemical determination of protein-bound iodine did not reveal significant changes at various times after the administration of radioactive inorganic iodide either in the hypothyroid or in the euthyroid state. In vivo measurements were substantially the same in both myxedema and euthyroidism. No accumu-

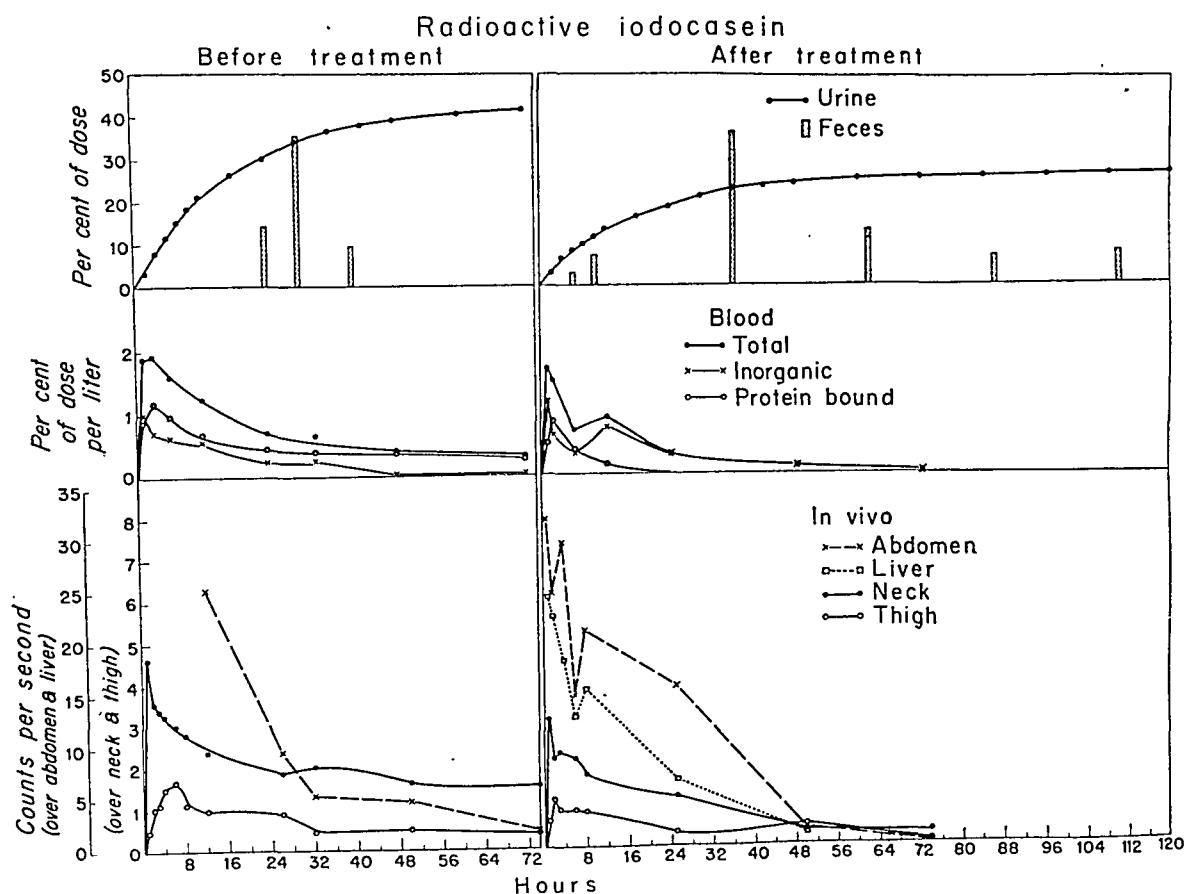


FIG. 3. The behavior of orally administered labeled iodocasein during myxedema and euthyroidism, as indicated by measurements of urinary and fecal excretion, blood levels, and in vivo observations.

lation of iodide was noted in any of the tissues, and in all cases the labeled iodide disappeared rapidly and uniformly from the various sites measured.

2. Behavior of labeled iodocasein.—Organic I^{131} (radioiodocasein) behaved quite differently from inorganic radioiodide, as comparison of Figure 3 with Figure 2 indicates. The I^{131} excreted in the urine during myxedema totaled 41.9 per cent of the radioactivity of the labeled dose in seventy-two hours, 43.6 per cent in 144 hours and 44.2 per cent in 216 hours. Within forty hours, 59.4 per cent of the dose had appeared in the

in blood were made according to the procedures used by McConahey and his associates (11). Total serum I^{131} , serum precipitable (protein-bound) I^{131} and nonprecipitable (largely inorganic iodide) I^{131} were estimated on serial samples after the ingestion of each tracer dose.

In vivo measurements of radioactivity were made serially over the thigh, neck, liver and epigastrium. The method for these procedures is described in detail elsewhere (12, 13).

C) *Chemical analyses*.—Chemical determinations of the plasma protein-bound iodine were kindly performed for us by Dr. Douglas Riggs, Boston, Massachusetts, to whom we are greatly indebted.

RESULTS

1. *Control date (the behavior of labeled inorganic iodide)*.—Figure 2 shows the distribution of radioiodine in the urine, feces, serum and various parts of the body, after the oral administration of a dose of radioactive inorganic iodide, before treatment when the patient was hypothyroid and after treatment when she was euthyroid. The results of the chemical determinations of the protein-bound iodine at various intervals after administration of the radioactive inorganic iodide are listed in Table 2.

TABLE 2. PROTEIN-BOUND IODINE AT VARIOUS INTERVALS FOLLOWING ADMINISTRATION OF RADIOACTIVE INORGANIC IODIDE

Hours	Hypothyroid	Euthyroid
	Micrograms per 100 cc. of plasma	
0	1.8	9.8
6	2.3	
12	2.0	12.0

When the patient was hypothyroid, 91.1 per cent of the inorganic radioiodide administered was excreted in the urine in seventy-two hours and 93.9 per cent in 142 hours. Over a period of five days, a total of 2 per cent of the dose appeared in the feces. Essentially the same distribution of I^{131} in urine and feces was noted when the patient was euthyroid. The curves of total serum radioactivity were essentially similar at both metabolic levels, with the exception that radioiodine disappeared somewhat more rapidly from the serum during the euthyroid state. Fractionation of the serum disclosed that in the hypothyroid state substantially all of the serum I^{131} was in the form of iodide until forty-eight hours after the administra-

fore or after control of the myxedema. However, the measurements over the abdomen, and particularly over the liver, were greatly increased within the first few hours, but decreased rapidly within twenty-four hours.

COMMENT

This study was planned so that the behavior of organically bound iodine could be compared with the behavior of the same amount of inorganic iodide at two levels of metabolism. These comparisons were made because of the possibility that the distribution and fate, not only of organically bound iodine, but also of inorganic iodide, might be different in hypothyroidism than in euthyroidism. In order to evaluate this factor so far as iodocasein was concerned it became necessary to study the distribution of iodocasein after the patient had been brought to equilibrium at a euthyroid level with nonradioactive iodocasein.

This program of study led to one finding with respect to inorganic iodide which for the moment remains unexplained. Approximately the same small proportion of the dose (4 per cent) remained unaccounted for in urine and feces in both hypothyroid and euthyroid studies with inorganic radioiodide. This small quantity undoubtedly reflects analytic errors, but nonetheless it probably reflects also a residue which is presumably distributed diffusely in other tissues of the body, as reported elsewhere (6). The thesis that it is not concentrated significantly receives some support from our failure to demonstrate localization by *in vivo* observations. Nevertheless, during myxedema a small quantity of I^{131} in the serum (0.04 per cent of the dose per liter) was found in precipitable form. No such precipitable fraction appeared in serum following administration of inorganic iodide when the patient was euthyroid. This finding could be interpreted as representing a minute and delayed synthesis of radioiodide into organic radioiodine compounds by tissues other than the thyroid (the so-called extra-thyroidal synthesis of thyroid hormone) which may be operative in myxedema and suppressed in the euthyroid state. The finding could also be attributed to synthesis of organic radioiodine in minute amounts by some residual thyroid tissue too small in amount to be detected by our *in vivo* methods, or it might represent an analytic artefact.

In other respects, the behavior of inorganic radioiodide at the two metabolic levels was consistent with the known effects of thyroid hormone on hemodynamics. Iodide disappeared more rapidly from the blood at a euthyroid level than at a myxedematous level (largely owing to a faster renal excretion rate), as indicated by more rapid appearance of radioiodine in urine, more rapid disappearance from blood and more rapid disappearance from peripheral tissues measured *in vivo*.

The metabolism of organic iodine, as contained in radioiodocasein, is

feces. During the euthyroid state, excretion of I^{131} in the urine amounted to 24.1 per cent in forty-eight hours, 25.7 per cent in seventy-two hours and 26.6 per cent in 144 hours. Over a 110-hour period, 51.7 per cent was excreted in the feces. In both experiments, most of the fecal radioactivity was found in a single stool excreted twenty-eight to thirty-two hours after administration of the radioactive iodocasein.

The maximal concentration of radioactivity observed in serum was very much less (about a third) after administration of radioiodocasein than after administration of inorganic radioiodide. At both metabolic levels, peaks of radioactivity in serum were reached in one hour, after which there was a progressive decline. In the hypothyroid state detectable amounts of radio-

TABLE 3. PROTEIN-BOUND IODINE AT VARIOUS INTERVALS FOLLOWING ADMINISTRATION OF RADIOACTIVE IODOCASEIN

Hours	Hypothyroid	Euthyroid
	Micrograms per 100 cc. of plasma	
0	1.5	12.4
6	4.6	20.6
12	3.8	12.9

iodine were present 144 hours after administration of the dose of iodocasein, but in the euthyroid state, very little was found after forty-eight hours. In the myxedematous state, one hour after administration of radioiodocasein, the radioiodine in the blood was found equally in the precipitable and nonprecipitable fractions. At forty-eight hours, 92 per cent was protein bound and at 144 hours all of the radioiodine in the blood appeared in protein-bound form. In the euthyroid state, however, 30.5 per cent of the radioiodine was protein bound in one hour, 57.3 per cent in two hours, 17.7 per cent in twelve hours and none in twenty-four hours.

Significant differences were noted by chemical determinations of the protein-bound iodine (Table 3) at varying intervals after ingestion of the labeled material. When the patient was myxedematous, administration of radioactive iodocasein resulted in values two to three times as great as the initial level of 1.5 micrograms per 100 cc. At the euthyroid level, similar studies disclosed almost a doubling of the protein-bound iodine six hours after ingestion of the dose, from an initial level of 12.4 micrograms per 100 cc.

After the administration of iodocasein, *in vivo* measurements did not reveal significant uptake of radioactivity in the thyroid area, either be-

the quantity recovered in the urine; 43.6 per cent in 144 hours in the first instance compared with 26.6 per cent in the same period in the second instance. The difference between these figures in effect constitutes "lost iodine," since no clue can be found from other observations which suggests what became of it. We were unable to find any technical error which would explain the difference and at the moment we have no explanation for it. Similar studies of other subjects may be necessary to determine whether this observation has significance.

A further observation of interest has to do with the correlation of protein-bound iodine and the clinical status of this patient. After therapy with iodocasein, in amounts considered equivalent to a maintenance dose of desiccated thyroid as determined by bio-assay in tadpoles, the protein-bound iodine was 12 micrograms per 100 cc. of plasma, a "hyperthyroid" level by most standards, despite the fact that the patient was clinically euthyroid. After four months of therapy with natural thyroprotein (0.1 Gm. of strong desiccated thyroid daily) the patient remained euthyroid clinically, and the protein-bound iodine had fallen to 4.0 micrograms per 100 cc. of plasma, a "euthyroid" level.

No exact explanation for this behavior can be given, inasmuch as one human assay does not permit deductions concerning the relative calorigenic potency of iodocasein versus desiccated thyroid. The following possibilities could be considered. Since the amount of organic iodine and of thyroxine iodine was three or more times greater in the dose of iodocasein than in that of desiccated thyroid, the equal metabolic effect could be due to poorer utilization of iodocasein than of desiccated thyroid. It will be recalled that more than half of the radioiodine of radioiodocasein was eliminated in the feces. Assuming that this represented half of the thyroxine radioiodine also, and (as seems likely) that the thyroxine content of iodocasein alone determines its calorigenic action, then the isocalorigenic effect could be partly explained on the grounds of inefficient absorption. To account now for the disparity in protein-bound iodine is not difficult, since iodocasein contains organic iodine in three or more times greater amounts in the form of nonthyroxine compounds than does desiccated thyroid. In the latter, the nonthyroxine iodine is essentially all in the form of diiodotyrosine, a substance that may be rapidly metabolized. In iodocasein, however, in addition to diiodotyrosine, there may exist other iodinated amino acids. The behavior of these could be quite similar to the behavior of such organic iodine compounds as gallbladder dyes (priodax) which, as is well known, are without calorigenic effect but are capable of elevating the level of protein-bound iodine for long periods.

however, much more complex than that of iodide. One must consider (14, 15) that soon after ingestion, some of the material might theoretically be absorbed intact; part might be broken up by digestive enzymes, yielding smaller fragments such as thyroxine and diiodotyrosine, as well as other amino acids and various peptide aggregates containing iodine, which would be more readily absorbed; lastly, depending on the completeness of digestion, some inorganic iodide might be liberated and absorbed. In addition to such complexities, the role of the liver must be considered, since it has been demonstrated that iodinated compounds may be secreted in the bile and also that liberation of inorganic iodide from such compounds may occur after passage through the liver. Such material as is excreted in this manner may be wholly or partly reabsorbed. Finally, some of the iodocasein or its fragments may be excreted from the digestive tract, either as unabsorbed material or as material that has been secreted into the bowel. The over-all picture may thus be a complicated one, consisting of digestion, absorption, intermediary metabolism, secretion and reabsorption.

The fractionation of the serum radioiodine into precipitable and non-precipitable fractions at various times after ingestion affords a general view of the sum of all such processes, but obviously cannot delineate any one of them. Any radioiodocasein absorbed intact as well as absorbed fragments of iodocasein, including diiodotyrosine and thyroxine, would in all probability appear in the precipitable or protein-bound fraction of serum, whereas liberated iodine in the form of inorganic iodide would appear in the nonprecipitable fraction. The analysis indicated in Figure 2 shows that iodocasein has been split rapidly, since within one hour an appreciable proportion of the serum radioiodine is in the form of inorganic iodide, and the remainder in organic form. This is also borne out by the chemical analysis for protein-bound iodine (Table 2). That the liver may contribute to this breakdown is suggested by the high concentration of radioactivity over this organ during the first twenty-four hours. The slow disappearance of inorganic radioiodide from serum over the course of three days would indicate a persistent release of iodide from iodocasein or its fragments, as the further fate of this inorganic radioiodide should follow the course outlined previously by the control study.

The fecal excretion of radioiodine after administration of the labeled doses of iodocasein amounted to more than half of the ingested material. Analysis of the radioiodine in feces showed that more than 90 per cent was in protein-bound form. Again it is not possible to determine the exact nature and origin of these compounds, but it would appear reasonable to regard this excretion as the sum of the processes mentioned previously.

The most notable difference between the behavior of radioiodocasein in the hypothyroid state and its behavior in the euthyroid state appeared in

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SUMMARY AND CONCLUSIONS

1. Iodocasein has been shown to correct the signs and symptoms of human myxedema. Although our observations do not permit an accurate appraisal of the relative potency of iodocasein as compared with natural thyroprotein, a considerably higher level of protein-bound iodine in blood was associated with maintenance of euthyroidism by iodocasein than by desiccated thyroid.

2. Comparative studies with labeled inorganic iodide (NaI^{131}) during myxedema and euthyroidism indicate that no demonstrable localization occurs in the athyrotic subject, nearly all of the iodide being accounted for in urine or feces. Furthermore, no transformation of iodide was observed, except possibly in the myxedematous state, in which small amounts of radioactivity were found in the protein-bound fraction of serum.

3. Comparative studies with labeled organic iodine (radioactive iodocasein) during myxedema and euthyroidism disclosed concentration of radioactivity over the gastro-intestinal tract and liver, high fecal and low urinary excretion. As indicated by blood levels of radioactivity and by fractionation studies, iodocasein was rapidly absorbed. Rapid and continuous degradation of the material was indicated by the early appearance and persistence of a significant labeled inorganic iodide fraction in serum.

4. Both iodide and iodocasein were disposed of more rapidly while the subject was in a euthyroid state than in a hypothyroid state.

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dried at 37–40° C. The final dry weights varied from 1.8 to 2.9 mg. (average 2.4 mg.). The net counts per minute (Table 1) varied from 8,150 to 8,740; the mean was 8,420. The standard deviation was 207.

TABLE 1. DUPLICABILITY OF COUNTS/MINUTE OBTAINED IN
10 IDENTICALLY PREPARED SAMPLES

Cup No.	Cup weight (Gm.)	Wt. of cup and dry residue (Gm.)	Wt. of dry residue (mg.)	Corrected cts./min.* ×10 ³
1	1.1237	1.1255	1.8	8.74
2	1.1481	1.1501	2.0	8.62
3	1.1440	1.1464	2.4	8.15
4	1.1089	1.1113	2.4	8.52
5	1.1238	1.1262	2.4	8.30
6	1.0896	1.0921	2.5	8.52
7	1.1292	1.1317	2.5	8.50
8	1.1292	1.1317	2.5	8.20
9	1.1323	1.1349	2.6	8.15
10	1.1420	1.1449	2.9	8.50

* Corrected counts per minute = counts per minute less background counts per minute. In this experiment background = 18 counts per minute.

Effect of pH

Three 10.0 cc. aliquots of an I¹³¹ solution were diluted to 1,000 cc. with distilled water and adjusted to pH 6.0, 7.0 and 8.6 respectively. Cups were prepared by the above described technique. Samples at pH 6.0 showed approximately 35 per cent less counts than samples at pH 8.6 (Table 2).

TABLE 2. EFFECT OF pH OF URINE ON MEASURED I¹³¹ ACTIVITY

Sample	pH	Corrected counts per minute ×10 ³	Average	Per cent alkaline samples
1	8.6	5.65	5.58	100
2	8.6	5.63		
3	8.6	5.45		
4	7.0	5.26	5.09	91
5	7.0	5.05		
6	7.0	4.92		
7	6.0	3.62	3.60	64.5
8	6.0	3.58		

COMPARATIVE VALUE AND ACCURACY OF MEASUREMENTS OF URINARY I^{131} BY BETA AND BY GAMMA RAY COUNTING*

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In our investigation of I^{131} to induce myxedema in euthyroid patients with angina pectoris and congestive failure (1) and in many studies of the effect of I^{131} in thyrotoxicosis and thyroid cancer, the retention of this agent in the thyroid has been estimated indirectly by measuring the urinary excretion (2-6) over a period of three days. The fraction retained in the body is assumed to reside mainly in the thyroid gland. In evaluating the accuracy of measurement of I^{131} in the urine, we observed a considerable decrease in counts per minute when equal amounts of I^{131} were added to urine as compared to distilled water. The purpose of this report is to evaluate this source of error and to describe the comparative advantages of two methods for measurement of I^{131} activity in urine.

METHODS

A. Measurement of Urinary I^{131} by Counting Beta Rays

A 1.0 cc. aliquot of urine containing I^{131} is pipetted into cleansed, weighed aluminum cups 3.1 cm. in diameter and 0.4 cm. high. Approximately 0.1 mg. NaI (1 drop 0.2 per cent NaI) and 2-3 mg. gelatin (1 drop 10 per cent gelatin) are added. Samples are evaporated to dryness at 37-40° C., and the time required for 4,096 counts determined, using an end-window Geiger-Mueller tube (mica window thickness of 3.2 mg./cm.²) in constant positional relationship to the cup. Counts per minute are calculated, and net counts per minute obtained by subtracting background counts. A standard is counted between every fourth sample to check the constancy of the Geiger-Mueller tube and scaling circuit as well as to afford the basis for calculating the amount of I^{131} in the sample.

Duplicability

One cc. of water containing I^{131} was pipetted into each of 10 cups; approximately 0.1 mg. NaI and 2-3 mg. gelatin were added, and the samples

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Effect of urinary solids

The effect of urinary solids on the measurement of beta radiation of I^{131} is shown in Figure 2. Cups were prepared, identical in respect to final volume, amount of I^{131} , pH, NaI-carrier and gelatin; they differed in that the solid content was varied from 4 to 75 mg. The variation in urinary solids was obtained by concentration and dilution of urine before the addition of radioactive iodine. One cc. of each mixture was pipetted into weighed cups, dried and counted as described above. The absorption approximates 12 per cent in a urine of average concentration.

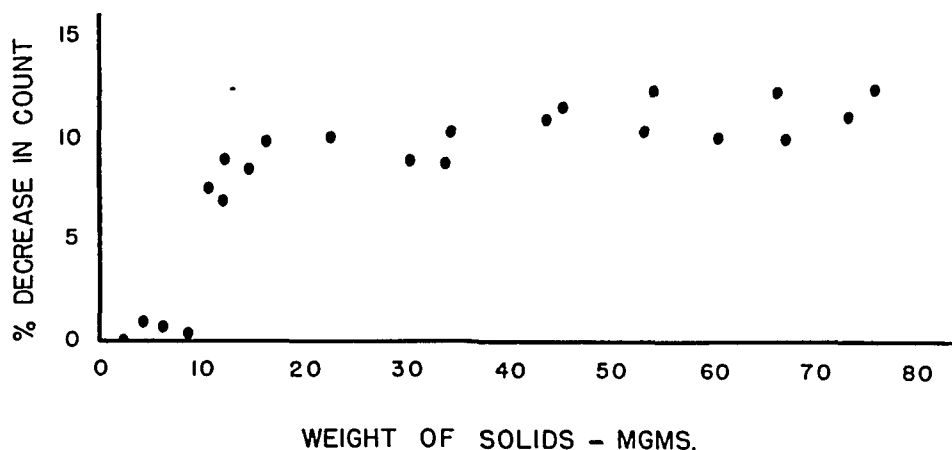


FIG. 2. Decrease in observed radiation due to mass absorption from urinary solids (see text for description).

To illustrate the use of Figure 2 in correcting counts for absorption by urinary solids, a characteristic experiment is shown in Table 3. The amount of I^{131} in the samples 630A and 630B was identical (Table 3). The dry weight of sample 630A was .0087 Gm. and of sample 630B, .0618 Gm. The average deviation at counting rates from 14,000 to 3,000 before correction was 10.5 per cent; and after correction for solid content, 1.9 per cent.

The effects of other chemical substances on the measurement of beta radiation

The effect of NaCl, urea, and gelatin on the measurement of beta radiation is illustrated in Figure 3. The increased loss of activity observed with these substances has not been explained; the appearance of the dried surface varied with each substance, and, in general, the surfaces in these experiments were rough and uneven. In contrast, the samples obtained with urine were smooth. It may be noted that correction for absorption as obtained with these substances would result in considerable error when applied to the experiments recorded in Table 3.

Description of the beta counting procedure for urine

Standards are prepared by diluting an aliquot of I^{131} in distilled water; the pH is adjusted to 8.0. One cc. is pipetted into each of three weighed

Studies at more alkaline pH were not desirable because of digestion of the aluminum cup.

Coincidence error

In counting high activity samples, considerable error is introduced due to coincidence loss. This is illustrated in the curve established by plotting the daily counts against the calculated curve of decay (Fig. 1). It is our

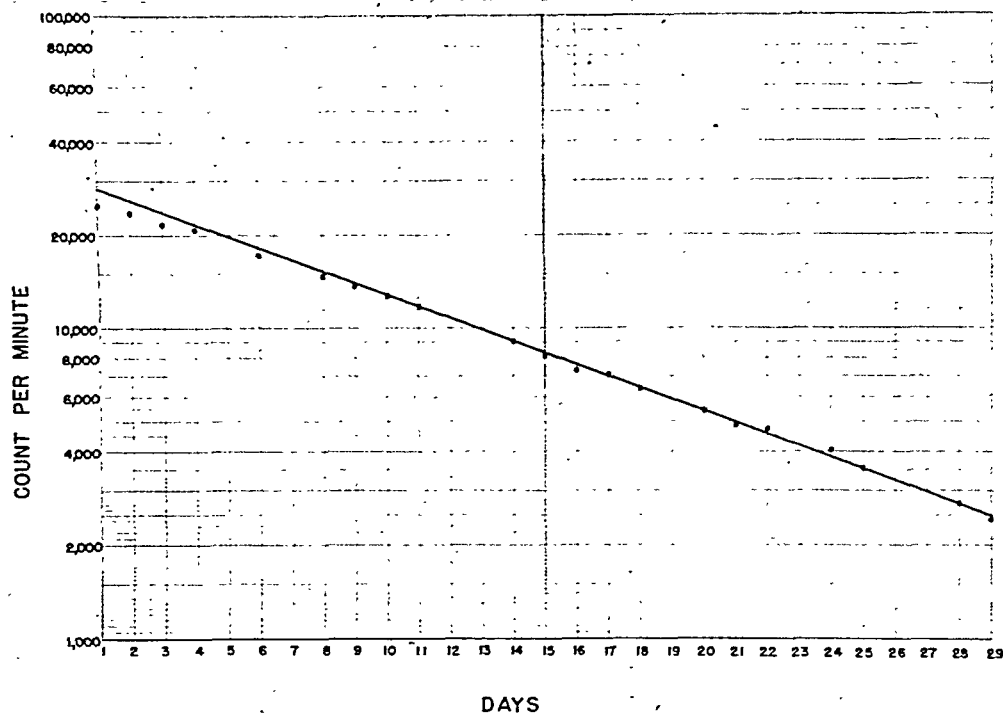


FIG. 1. *The loss in observed radiation due to coincidence counts.*

I^{131} standards, in triplicate, were prepared as described under beta counting procedure for urine. The dots are the observed values. The calculated counts/minute are represented by the line. On days 1-3, with activities greater than 20,000 counts/minute, the observed radiation is 11 per cent less than the calculated counting rate.

practice to recount, at a later date, samples which are more active than 15,000 cts./min. As pointed out in the section on gamma counting, a correction factor for loss due to coincidence counts may be established for the Geiger-Mueller tube by the usual technique of counting two sources individually, then together and computing the per cent decrease in observed counts.

cups. Approximately 0.1 mg. NaI (1 drop 0.2 per cent NaI solution) and 2–3 mg. gelatin (1 drop 10 per cent gelatin) are added to each cup. The samples are evaporated to dryness; the dried samples weighed, and determinations of the radiation made as described above. The same procedure is carried out in triplicate on 1.0 cc. samples of the patient's urine, adjusted to pH 8.0. Under these conditions the triplicate samples agree within 3

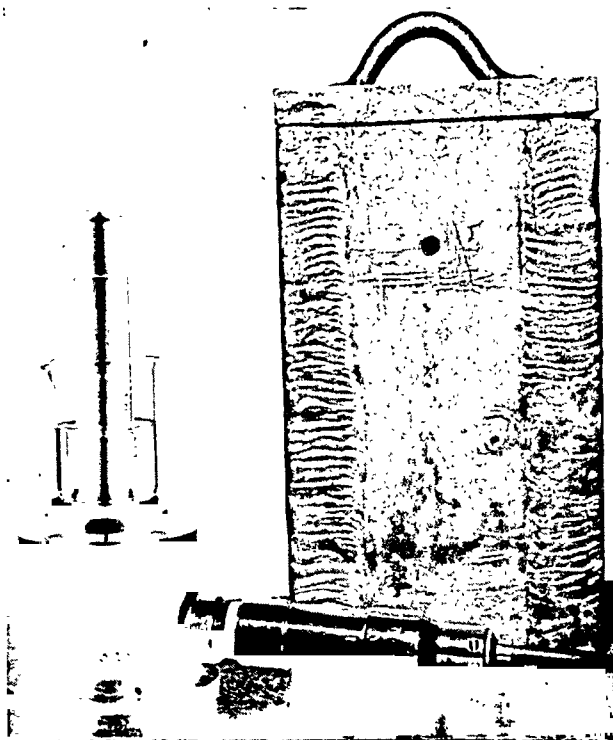


FIG. 4. The Geiger-Mueller tube, covered by a stainless steel shell, and the Marinelli beaker for measurement of urinary I^{131} by counting gamma rays.

per cent. The counts are corrected for background and for internal absorption by use of the curve shown in Figure 2. The per cent excretion

$$\frac{\text{cc. urine volume} \times \text{corrected counts/min./cc.}}{\text{urine} \times \text{dilution} \times \text{factor for internal absorption} \times 100}$$

$$\frac{I^{131} \text{ dose in cc.} \times \text{corrected counts/min./cc.}}{\text{standard} \times \text{dilution} \times \text{factor for internal absorption}}$$

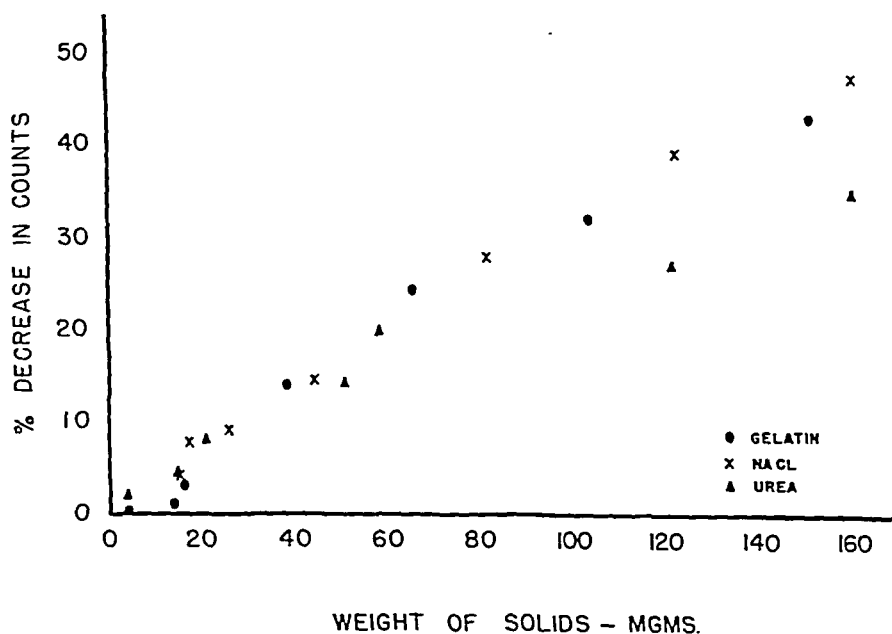
Under the conditions of our technique, with sufficiently active samples, the dilution of samples to contain less than 10 mg. total solids obviates the necessity for the use of a correction factor for internal absorption.

B. Measurement of Urinary I^{131} by Counting Gamma Rays

The above described method is accurate but laborious. Furthermore because of the necessity for slow drying, results cannot be obtained before

TABLE 3. THE EFFECT OF URINARY SOLIDS ON OBSERVED RADIATION
(see text for description)

Date of count	Counts uncorrected for absorption			Counts corrected for absorption		
	Sample 630A counts/min.	Sample 630B counts/min.	Per cent diff.	Sample 630A counts/min.	Sample 630B counts/min.	Per cent diff.
1-14	14040	12430	11.3	14170	14050	.8
1-15	12570	11570	8.0	12710	13080	2.8
1-16	11710	10840	7.4	11830	12240	3.3
1-17	10950	9770	10.9	11050	11030	.0
1-20	8500	7730	9.5	8590	8730	1.7
1-21	7860	7020	10.8	7940	7940	.0
1-22	7200	6370	11.6	7270	7200	1.0
1-23	6510	5950	8.6	6580	6720	2.2
1-24	5980	5260	12.0	6050	5940	1.8
1-26	5140	4480	12.7	5200	5070	2.4
1-27	4700	4030	14.2	4750	4550	4.3
1-28	4380	3780	13.7	4430	4270	3.5
1-29	3820	3480	8.9	3850	3930	1.9
1-30	3630	3270	9.8	3670	3691	.6
1-31	3250	2990	7.9	3290	3370	2.4

FIG. 3. The effect of sodium chloride, urea and gelatin on the measurement of beta radiation from I¹³¹ (see text for description).

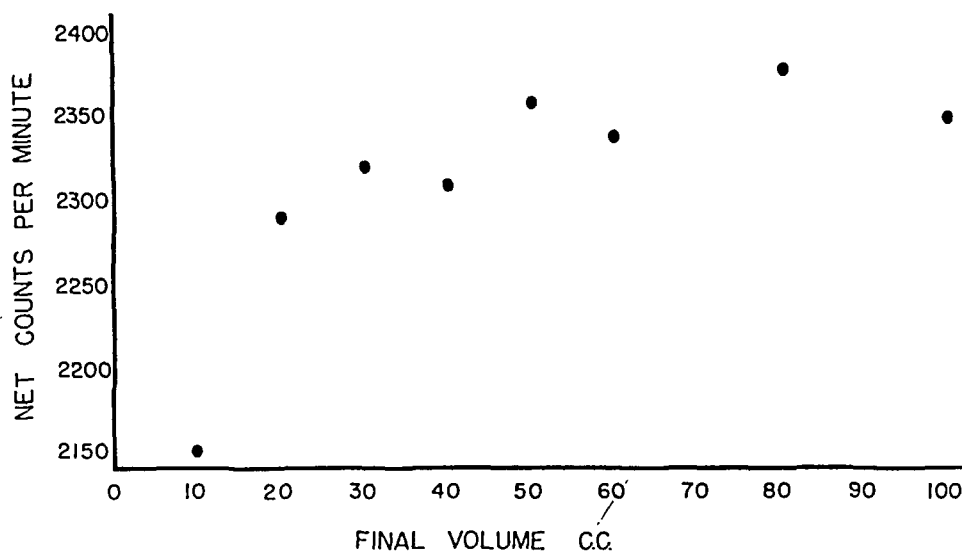


FIG. 5. The effect of volume on observed gamma radiation (*see test for description*).

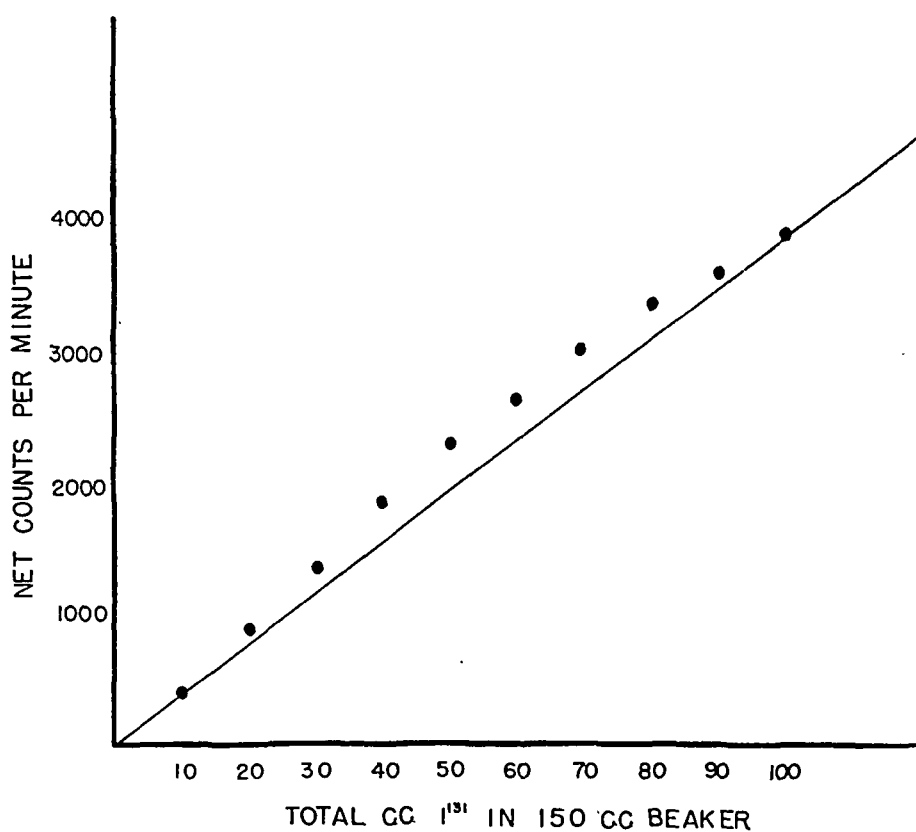


FIG. 6. The effect of adding 10 cc. increments of an I¹³¹ standard on observed radiation (*see text for description*).

twenty-four hours. After consultation with Mr. L. D. Marinelli and Miss Ruth Hill of the Memorial Hospital, New York, we set up the gamma counting technique employed by them (7). The Sylvania tube, GG 305, covered by a stainless steel shell and the Marinelli beaker are pictured in Figure 4.¹ Shielding is accomplished by a lead housing.

Duplicability

Fifty cc. aliquots of an I^{131} standard were pipetted into each of ten Marinelli beakers. The net counts per minute varied from 1,910 to 2,020, the mean being 1,950 (Table 4). The maximum variation between samples was 5 per cent.

TABLE 4. GAMMA COUNTS PER MINUTE OBTAINED IN 10 IDENTICALLY PREPARED SAMPLES

Beaker No.	Counts/min.	Background	Corrected counts/min.
1	1990	70	1920
2	2040	70	1970
3	1990	70	1920
4	2040	70	1970
5	1980	70	1910
6	2000	70	1930
7	2090	70	2020
8	2010	70	1940
9	2050	70	1980
10	2010	70	1940

Effect of volume on observed radiation

Figure 5 shows the variation obtained when net counts/min. are plotted against the total volume in cc. Two cc. of an I^{131} standard was pipetted into a Marinelli type beaker. Eight cc. of water was added and 4,096 counts made. The volume was increased by increments of 10 cc. of water and 4,096 counts determined at each volume change. With final volumes of 20 to 100 cc., there was a maximum variation of 4 per cent in counts per minute.

The effect of adding 10.0 cc. increments of an I^{131} standard is illustrated in Figure 6. There was a loss in observed radiation in volumes under 30 cc. and above 80 cc. For 10 cc. the loss, when compared with 50 cc., was 7.5 per cent. For 100 cc., when compared with 50 cc., the loss was 12 per cent.

¹ Made by Tracerlab, Inc. Boston, Mass. We wish to thank Dr. F. Henriques for his cooperation and help.

COMMENT

The data presented in Table 7 and its statistical evaluation show that the gamma and beta procedures give comparable results. These methods have been in use in our laboratory for the last year and a half, (1, 11, 12) and some indications of the advantages and limitations of each method for measuring I^{131} in urine have been gained.

TABLE 7. COMPARISON OF BETA AND GAMMA METHODS FOR DETERMINATION OF URINARY EXCRETION OF I^{131}

Case	Beta method Per cent	Gamma method Per cent	Per cent difference
1	.41	.41	0
2	.42	.41	2.4
3	.48	.50	3.8
4	.71	.72	.2
5	.76	.81	6.2
6	1.3	1.23	5.3
7	4.1	3.4	17.1
8	4.9	5.7	14.1
9	8.7	9.1	4.3
10	9.7	9.1	6.1
11	10.3	10.8	4.5
12	11.6	11.1	4.2
13	12.6	13.1	3.7
14	15.5	15.3	1.2
15	21.9	21.2	3.2
16	22.9	23.7	3.4
17	33.0	34.8	5.1
18	64.6	64.8	.3

The beta method is more sensitive, giving counts per microcurie approximately 250 times those obtained by the gamma method. In man, following the oral administration of 150 $\mu\text{c.}$, the amount of I^{131} in various tissues and blood is minute, approximating 0.005–0.002 $\mu\text{c.}$ per cc. of total serum (13). The gamma technique we have described cannot measure accurately less than 0.25 $\mu\text{c.}$ Thus, when amounts of radiation are small or must be determined in blood (13) or tissue (12), the beta method is the method of choice.

In determination of urinary excretion when larger amounts of I^{131} are present, the gamma counting method is simple and results can be obtained promptly. Mass absorption and pH within the range usually observed in urines do not have to be considered. The use of the beta method for urines with activities larger than 1 microcurie per twenty-four hour excretion is cumbersome. The pH must be adjusted to the alkaline side, and mass

These experiments indicate the desirability for comparing equal volumes of the unknown and standard.

Coincidence error

The recovery time of the gamma tube was calculated according to the equation

$$(8) (9): \quad RT = \frac{2(N_1 + N_2 - N_{12})}{(N_1 + N_2) (N_{12})}$$

N_1 and N_2 equal net counting rates per second of similar sources counted individually; N_{12} equals these two sources counted simultaneously. N_1 was counted first, then N_2 was placed and the counts from N_{12} determined. N_1 was removed and N_2 counted. In this tube, the recovery time was 840–1,300 microseconds (Table 5). The correction factor for loss due to coincidence or per cent loss at varying counting rates is shown in Table 6.

TABLE 5. RESOLVING TIME OF GAMMA TUBE

Expt.	Source N1 (counts per minute)	Source N2 (counts per minute)	Source N12 (counts per minute)	Resolving time, micro seconds
1	3820	3870	7300	840
2	4110	3950	7410	1300

TABLE 6. COINCIDENCE ERROR

Expt.	Observed counting rate/min.	Calculated counting rate/min.	Factor
1	3610	3650	1.01
2	4980	4970	1.00
3	7410	8060	1.09
4	9910	11070	1.11
5	13600	15000	1.11
6	15500	17600	1.14

C. Comparison of Beta and Gamma Method to Determine Urinary excretion of I^{131}

In 18 subjects, the urinary excretion after an oral dose of I^{131} was determined by both the beta and gamma counting procedures described above. The per cent excretion in twenty-four hours varied from 0.41 to 64.6 (Table 7). The average difference between the two methods was 4.72 per cent with a range of 0 to 17.1 per cent; the median value was 3.8 per cent. The standard deviation was 4.37. The standard error was 0.73 and $P(10)$, 0.78.

URINARY PREGNANEDIOL DETERMINATION AS A TEST OF PREGNANCY*

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IN 1936 Venning and Browne (1) described the isolation of a water-soluble pregnanediol complex from human pregnancy urine. In 1937 Venning, Henry and Browne (2) described the measurement of this complex and the same year Venning (3) published a gravimetric method for determination of sodium pregnanediol glucuronide in urine, and presented evidence of its metabolism from progesterone. Venning and Browne (4) and Venning, Henry and Browne (5), using this procedure, reported the presence of significant amounts of the complex in urine from women in the luteal phase of the menstrual cycle. Subsequently, these workers and many others using the same procedure, investigated the urinary excretion of sodium pregnanediol glucuronide in pregnant and nonpregnant women (6-18). Urinary sodium pregnanediol glucuronide levels were reported to be of value in the diagnosis of pregnancy, of threatened abortion, and determination of the date of ovulation, all states reflecting a change in luteal activity. Since Venning's method has been reported inaccurate for small amounts of sodium pregnanediol glucuronide (19), its application to subjects during the menstrual cycle is of doubtful value; moreover, the estimation then requires the collection of a 24-hour and preferably a 48-hour specimen of urine, with increasing opportunity for hydrolysis of the water-soluble complex.

In 1944 Guterman (20) published a qualitative colorimetric method for determination of free pregnanediol, based on the methods of Astwood and Jones (21) and of Talbot *et al.* (22). The procedure was extended and modified in subsequent papers by the same author (23, 24, 25), and developed on a quantitative basis, applicable as a diagnostic aid for pregnancy and threatened abortion.

The Guterman procedure has been widely investigated and both the analytical technique and the diagnostic value have been criticized. McCormack (26), using a slightly modified technique and applying the results qualitatively to a group of 304 patients, found the accuracy of diagnosis by the Guterman test comparable to that of the Friedman modification of the Aschheim-Zondek test. Morrow and Buena (27), following the Guter-

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absorption may be a considerable factor. It is important to emphasize that in the dried sample the possibility of loss from handling exists. We, therefore, prepared all samples for the beta procedure in triplicate. The necessity of weighing cups, as well as the other factors mentioned above, militate against the usefulness of the beta procedure for the routine determination of urinary excretion.

SUMMARY

1. Two procedures used for determination of urine I^{131} radiation are described. The importance of mass absorption as a possible source of error and its correction is pointed out in the measurement of I^{131} activity by counting beta rays.

2. A study of the urinary excretion of I^{131} by the described beta and gamma counting procedures yielded agreement within five per cent.

3. The advantages and limitations of the two procedures are briefly discussed and the indications for the use of each are given.

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- A. *Hydrolysis and extraction.* In accordance with the suggestion of Sommerville *et al.* (30), 10 ml. of concentrated hydrochloric acid was added to the urine/toluene mixture at the point of boiling, to prevent unnecessary exposure of the pregnanediol to strong acid. To provide more thorough separation of pregnanediol three toluene extractions were carried out in place of only one.
- B. *Precipitation of impurities.* In the Guterman procedure toluene/water emulsions caused some difficulty but could be avoided by prolonging the time required for separation and by drying any residual emulsion with anhydrous sodium sulphate, which was then removed by filtration. The sodium sulphate residue was washed twice, on the filter paper, with small amounts of toluene.
- C. *Precipitation of pregnanediol.* Whereas Guterman allowed the acetone/sodium hydroxide solution containing pregnanediol to stand for one hour at 5° C, it was found advantageous to lengthen the time to sixteen hours at a temperature of 10° C.
- D. *Isolation of pregnanediol.* The precipitated pregnanediol, on the fritted glass filter, was washed with 10 ml. of petroleum ether to remove 17-ketosteroids, as suggested by Davis and Fugo (33).
- E. *Development and measurement of color.* The pregnanediol, finally separated, was dissolved in 5 ml. of pure ethanol. Convenient-sized aliquots (0.2–1.0 ml.) of the solution were pipetted into 25 ml. test tubes, the ethanol evaporated in boiling water, the tubes allowed to cool, and 10 ml. of concentrated sulphuric acid buretted into each. In contrast with the experience of Sommerville *et al.* (30), it was found desirable to allow forty-five minutes for development of maximum color. The color value was found to be constant during a 45–60 minute period after addition of the acid. Color intensity was read in a Coleman No. 11 Universal spectrophotometer, at a wave length of 430 m μ , against a blank of concentrated sulphuric acid. Calibration curves were prepared daily, using a standard solution of sodium pregnanediol glucuronide in 90 per cent ethanol. Experiments in which pregnanediol was separated from the sodium glucuronide indicated that the color developed was due entirely to the pregnanediol. The conversion figure for expressing sodium pregnanediol glucuronide in terms of pregnanediol was derived directly from the molecular weights of the two compounds and was 0.597.

To avoid possible contamination from rubber or cork stoppers, standard taper glassware was used for all hydrolysis and extraction procedures.

All reagents were tested for possible contribution to color formation and were found to be without effect. Six specimens of male urine, subjected to the complete procedure, gave zero readings. The method was checked by recovery experiments, with the results shown in Table 1.

The results of the above recovery experiments tend to substantiate the statement of Sommerville *et al.* (30) that the procedure is of doubtful accuracy for small amounts of pregnanediol.

Collection of urine specimens. It is generally difficult to obtain 24-hour specimens of urine; for convenience, first morning specimens have obvious advantages. It seemed useful to determine whether the estimation of pregnanediol in such specimens was of diagnostic value. All results reported here have been obtained with first morning specimens. Urine was preserved by saturation with benzoic acid, a substance which does not in-

man procedure, also with slight modifications and using an artificial color standard, found the test of doubtful value owing to the large number of false positives in women in the luteal phase of the menstrual cycle. Reinhart and Barnes (28) applied the Guterman test to 130 patients and reported a positive and negative error of 25 per cent. They suggested that this might be due to individual variation in progesterone metabolism. The results of the two latter groups have been criticized by Guterman (25) because Morrow and Buena used an artificial color standard of potassium dichromate and a wave-length of 530–570 $m\mu$, whereas Reinhart and Barnes employed an artificial color standard of potassium dichromate, not comparable in intensity with that given by 0.4 mg. of pregnanediol. Huber (29) found Guterman's method highly satisfactory for extraction and for removal of acidic and phenolic compounds but preferred a chromatographic absorption to the precipitation from acetone/aqueous sodium hydroxide solution, and reported that gravimetric determination of the purified pregnanediol was superior to a colorimetric procedure. Sommerville, Marrian and Kellar (30) suggested that the Guterman method was liable to give erratic results at low levels of pregnanediol excretion and fictitiously high results in the presence of abnormally large amounts of cholesterol or 17-ketosteroids. These authors offered an alternative method of purifying and isolating the pregnanediol; the procedure for development of color was also modified, being carried out at 25° C for twenty minutes only. Bender (31) investigated pregnanediol excretion of 100 cases, diagnosed clinically as threatened abortion, and concluded that the abortion rate could be reduced by about 25 per cent if progesterone therapy was given to those showing a negative or weakly-positive Guterman test. Merivale (32) reported only a 57 per cent accuracy for diagnosis of pregnancy by the Guterman test.

The object of the present study was to evaluate the determination of urinary pregnanediol excretion as a diagnostic test for pregnancy. Since an appreciable amount of pregnanediol is excreted by women in the luteal phase of the menstrual cycle, it was decided first to investigate the excretion of this substance by a group of nonpregnant females in both phases of the cycle. The two points of interest in such levels were:—

1. Is there a statistically significant difference in pregnanediol excretion in the follicular and luteal phase of the menstrual cycle?
2. Is the excretion in early pregnancy sufficiently different from that of nonpregnant females, in the luteal phase of the menstrual cycle, to enable the determination to be used as a diagnosis of pregnancy?

PROCEDURE FOR THE ESTIMATION OF PREGNANEDIOL

The quantitative procedure of Guterman (25) was used as a basis, but the following modifications were found to be advantageous:—

luteal phase of the menstrual cycle, there was overlapping of individual levels. The level which is to be considered as diagnostic of pregnancy is debatable. Guterman first fixed the "positive" level as 1.0 mg. of pregnanediol per 100 ml. of urine, or 15–20 mg. total for 24-hour excretion; he later proposed 0.4 mg. per 100 ml. or 6–8 mg. per 24-hour specimen. Both levels were selected by Guterman on the basis of personal judgment, arrived at by a study of the observed values. It seemed desirable to have a

TABLE 2. URINARY PREGNANEDIOL VALUES

Group	Clinical condition	Number of subjects	Pregnanediol concentration, mg./100 ml. of urine	
			Range	Mean and standard deviation
<i>Control:</i> School of Hygiene Staff and T.G.H. Dietitians	Follicular phase of menstrual cycle	38	0.07–0.51	0.22 ± 0.119
<i>Control:</i> School of Hygiene Staff and T.G.H. Dietitians	Luteal phase of menstrual cycle	38	0.06–1.81	0.42 ± 0.344
<i>Clinical:</i> Outpatients, Pre-Natal Clinic T.G.H.	Pregnant 4–12 weeks since last menstrual period	52	0.30–9.50	2.44 ± 1.864

critical level which would be independent of subjective opinion and which would be determined by statistical treatment. A critical level of this type has been calculated in the following way:—The actual pregnanediol levels in the three series reported above are not normally distributed but the logarithms of the values are approximately normally distributed. A critical level, lying between the two means, was selected so that the probability of a pregnant woman being classified as "nonpregnant" would be equal to the probability of the opposite type of error, assuming a normal distribution of logarithms of the observed values. The estimated error for either type of mistaken diagnosis, arrived at from this critical level, would be about 12 per cent. In other words, the theoretical accuracy of the test would be about 88 per cent.

Using the two critical levels proposed by Guterman and that which has been calculated from our data, all subjects have been classed as "pregnant" or "nonpregnant" depending on whether the observed value was

TABLE 1. RECOVERY OF PREGNANEDIOL

Test vehicle	Sodium pregnanediol. glucuronide added before acid hydrolysis, expressed as mg. pregnanediol	Per cent recoveries: mean of 3 determinations
100 ml. water	2.5	96
	1.0	76
	0.5	70
100 ml. male urine	5.3	110
	2.4	91

terfere with the determination of pregnanediol. Estimations were made as soon as possible after collection.

CLINICAL MATERIAL

1. *Control group.* To arrive at a "base-line" of pregnanediol excretion by normal, nonpregnant females, a group of 44 persons (age 19 to 36 years) was studied. The estimated length of the menstrual cycle was recorded, together with the actual length of two consecutive cycles. A first morning specimen of urine was collected on the ninth, tenth or eleventh day, and the twentieth, twenty-first or twenty-second after the beginning of menstruation. A study of the recorded cycles allowed an estimation of the time of ovulation (approximately fourteen days prior to the beginning of the second menstrual period). Only those individuals whose second specimen could be assumed to be postovulatory were included in the final group which totalled 38 persons.
2. *Clinical group.* A group of 59 women, whose menstrual period had been missed for four to twelve weeks and who were attending the Pre-Natal Clinic, Toronto General Hospital, furnished first morning specimens of urine. Of this number, 52 were pregnant.

RESULTS

Urinary pregnanediol levels are summarized in Table 2. Using Fisher's "t" test, the t values for the differences between the means of the two control levels was 3.1, a figure which is highly significant. The distribution of the logarithms of pregnanediol values is shown in Figure 1.

Use of pregnanediol levels for the diagnosis of pregnancy. Although there was a marked difference between the mean values of pregnanediol concentrations in urine specimens from pregnant women and from those in the

above or below the particular critical level. The classification thus obtained was compared with the clinical information for each subject, to determine the accuracy of diagnosis given by the pregnanediol test. The percentages of accuracy for the three series of results, calculated from each critical value, are given in Table 3.

DISCUSSION

The reliability of the diagnosis of pregnancy by the determination of pregnanediol depends primarily on whether the amount of the substance excreted during pregnancy is different from that in the nonpregnant state. The results reported here show that there is considerable overlapping between levels observed in the luteal phase of the menstrual cycle and those found in pregnancy. If there were a distinct gap, the use of the pregnanediol test would be simple. Since that is not the case, a critical level has to be selected and it is advisable to do so by a procedure which is free from subjective error. Statistical treatment of our data yielded a critical level which, theoretically, has an error of 12 per cent. Actually, by the application of this level to the data reported above, 8 per cent of nonpregnant subjects would be classified as "pregnant," and 13 per cent of pregnant women would be classified as "nonpregnant." It should be noted that evaluation of our data on this basis gave more false negatives than false positives.

Various statements of the reliability of biologic tests for pregnancy have been made. In this laboratory the accuracy of the Friedman modification of the Aschheim-Zondek test, conducted on rabbits, was found to be about 98 per cent (34). Two rabbits were used for each test and the test was repeated if the two animals gave discordant results. Guterman reported that the Friedman test was 87 per cent accurate (23). The female South African frog (*Xenopus laevis*) test has been said to be 99.6 per cent reliable for diagnosis (35). It is likely that the pregnanediol test cannot be considered as accurate as the established biologic procedures, but the chemical method has the advantage in not requiring the maintenance of animals. In our opinion it is a useful procedure, particularly if it is applied with the proviso recommended by Guterman, that the test be considered positive only after at least one missed menstrual period. We would add an additional proviso, that the result be evaluated in terms of a statistically obtained critical level.

The pregnanediol test might be even more reliable if 24-hour specimens were used, but, in practice, these are difficult and often impossible to obtain. A previous report from this laboratory has indicated a relation between the concentration of 17-ketosteroids in the urine of normal males and the specific gravity of the urine (36); no similar relation was found for pregnanediol.

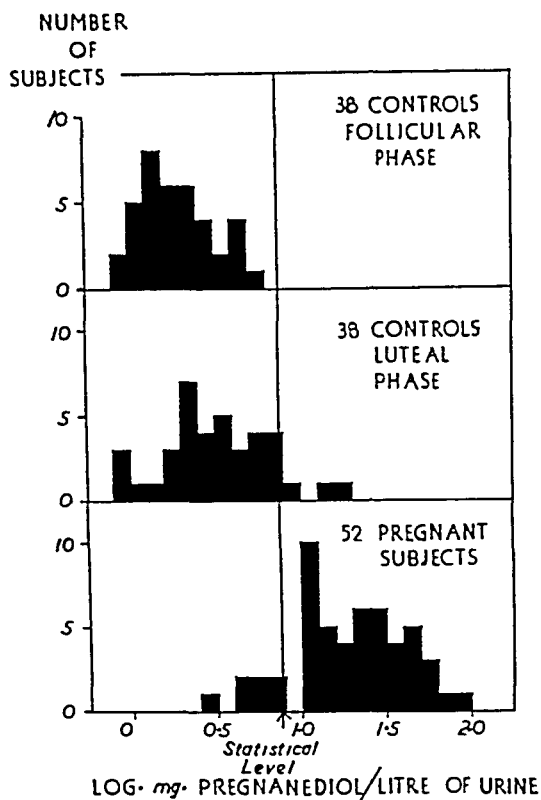


FIG. 1. Distribution of logarithms of pregnanediol levels in urine. The "statistical level" is the value which has been selected statistically, to be used for diagnosis of pregnancy.

TABLE 3. DIAGNOSTIC VALUE OF PREGNANEDIOL DETERMINATIONS

Group	Number of subjects	Correct diagnosis		
		Guterman's level 1.0 mg./100 ml.	Guterman's level 0.4 mg./100 ml.	Statistical level 0.77 mg./100 ml.
		Per cent	Per cent	Per cent
<i>Control:</i> follicular phase	38	100	87	100
<i>Control:</i> luteal phase	38	95	63	92
<i>Clinical:</i> early pregnancy	52	87	98	87

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SUMMARY AND CONCLUSIONS

A modification of the Guterman procedure has been developed and used for the determination of urinary pregnanediol. The procedure is probably not accurate for amounts less than 0.5 mg./100 ml. of urine; figures of this order have been reported here but have been used with reservation. Determinations were made on morning specimens of urine preserved with benzoic acid, from 38 nonpregnant women in both phases of the menstrual cycle, and from 52 women who were four to twelve weeks pregnant. Although there was overlapping of individual values between the two phases of the menstrual cycle and between the luteal phase and early pregnancy, the means of the three sets of values were significantly different. A statistical method for selecting a critical "positive" level is proposed; using this level, the diagnosis of pregnancy from pregnanediol concentration in the urine was theoretically 88 per cent accurate. In the cases reported there were 8 per cent false positives and 13 per cent false negatives. Although the procedure is not as reliable for diagnostic purposes as are several biologic methods, it is considered useful, particularly when animal facilities are not available.

Acknowledgments

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PSEUDOHYPOPARATHYROIDISM

REPORT OF A CASE WITH LATE MANIFESTATIONS

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IN 1942 Albright (1) and his co-workers first described a condition resembling hypoparathyroidism in which there appeared to be no deficiency of the parathyroid secretion but a failure of the body end-organs to respond to the hormone. Three patients were reported who presented certain characteristic physical features with histories of convulsions or other symptoms of parathyroid deficiency, and in whom the results of chemical studies were identical with those of hypoparathyroidism except for complete lack of response to administered parathyroid hormone. In one patient a biopsy revealed apparently normal parathyroid tissue. It was postulated that a normal amount of parathyroid hormone was secreted but that the tissue end-organs were refractory to the hormone. The name "pseudo-hypoparathyroidism" was given to this condition. It has also been called an example of the "Seabright-bantam syndrome." The Seabright bantam is a species of fowl in which the male shows female plumage and comb. Normal testes have been demonstrated, and this species has been considered the classic example of end-organ refractoriness to a normal amount of circulating hormone. Review of Morgan's original papers (2, 3) on the Seabright bantam leaves some doubt as to whether this bird represents a true example of end-organ refractoriness. It was conclusively shown that following castration the male bantam developed male plumage and comb. This suggests an endocrine-inhibiting mechanism rather than true end-organ refractoriness. Perhaps the term "Seabright-bantam syndrome" should be dropped but, nevertheless, the concept of a refractory end-organ producing a picture resembling a glandular deficiency is valid. A simpler example is the beardless American Indian who has a normal amount of testosterone.

Four additional cases of pseudohypoparathyroidism have been reported; one by Sprague (4) and his co-workers; two by Selye (5); and one by Peterman and Garvey (6).

Because of the rarity of the condition and because of the important

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forty years. One year before admission he had first noticed a tremor of the hands and a shuffling type of gait. The patient had had a normal sex life, was married and the father of two apparently normal children. He had never received any endocrine treatment.

Physical examination revealed a short, stocky male, whose gait was slightly propulsive, with the elbows held in slight flexion. Body measurements were as follows: weight 145 lbs., height 59½ in., span 57 in., from symphysis to vertex 31 in., from symphysis to floor 28½ in., circumference of the head 23 in., circumference of the chest 37 ¾

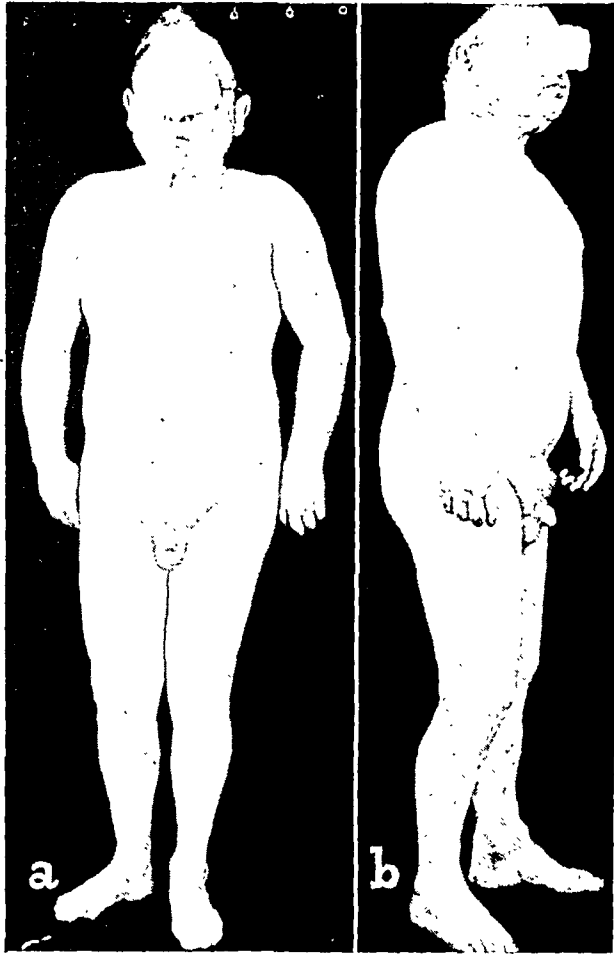


FIG. 2 (a and b). The patient at the time of admission, showing the characteristic physical appearance as described in the text.

in., and circumference of the abdomen 36 in. The blood pressure was 112/75. Temperature was 98.6°, pulse 72, and respiration 18. The face was round, the neck short and thick, and the limbs appeared short in relation to the trunk (Figs. 2a and 2b). Hair distribution and skin texture were normal. The pupils were round, equal, and reacted normally. Bilateral early lens opacities were present beneath the anterior and posterior capsule. The fundi were normal. The ears, nose, and throat were not remarkable. Dental examination revealed all the teeth to be small, especially the bicuspid. There was a marked overlapping and all the teeth showed advanced abrasion. The left lower second bicuspid and the upper right cuspid were unerupted, although roentgenograms showed no evidence

theoretical implications of a condition of end-organ defect, such cases deserve further study. The following case is presented as an example of pseudohypoparathyroidism diagnosed late in life, and showing some of the late manifestations of the disease:

CASE REPORT

V. S. (MCV #37753), a 49-year-old white male, was admitted to the Medical College of Virginia Hospital on April 8, 1948, complaining of pains in the extremities.

Family history revealed that one brother died of diabetes and one of tuberculosis. There was no other history of endocrine disorders, and the patient's parents had been normal as far as he knew.

The patient was born at term. No other details of the pregnancy were known. He was always a "fat" baby, having weighed 45 pounds at the age of nine months (Fig. 1). He



FIG. 1. The patient at age nine months, weight 45 pounds. Note that the fat, rounded head and short stubby extremities are apparent at even this early age.

had "never grown as other children," always being short and fat, with a round face and short limbs. Between the ages of 17 and 20 he had convulsive seizures which varied from three to four daily to once a month. He was never incontinent with these seizures. No cause was found, and he "outgrew" them and had no recurrence, with the possible exception of one fainting spell at the age of 37. At the age of 25 he first noticed painless firm, warty nodules on the fingers which gradually increased in size and number. At the age of 28 he noticed pain and stiffness in the right elbow. This was intermittent, but during the following years he developed increasing stiffness and shooting pains in both shoulders, elbows, neck, back, hips, and feet. These had finally incapacitated him a few months before his admission.

The patient had worn glasses for forty years, but during recent months had noticed progressive blurring and dimness of vision. He had had mild periodic asthma for about



FIG. 4. Roentgenogram of the skull showing calcifications in the region of the basal ganglia.



FIG. 5. Roentgenogram of the right leg showing extensive soft tissue calcinosis.

of significant impaction. These teeth normally erupt between the ages of 10 and 13 years. The lungs showed moderate emphysema and scattered expiratory wheezes. The heart was normal and the abdomen not remarkable. The genitalia were small but normally developed.

Over the first three fingers of both hands, over the dorsum of both feet, and attached to the Achilles tendons there were numerous stony hard subcutaneous nodular plaques. These were non-tender and immobile, being apparently firmly attached to the deeper structures (Fig. 3). There was limitation of motion of the shoulders, spine, hips, and feet.



FIG. 3. Palmar view of the left hand showing the subcutaneous calcifications.

Neurologic examination was not remarkable except for a gross "pill-rolling" type of tremor of the forearms and hands, more marked on the right, present at rest, but disappearing with intentional movements. Chvostek and Trousseau signs were absent.

Laboratory studies showed normal blood counts and urinalyses. The blood chemistry was as follows: serum calcium 6.8 mg. per cent, serum inorganic phosphorus 4.5 mg. per cent, total serum protein 7.7 Gm. per cent with albumin 4.3 Gm. and globulin 3.4 Gm., serum cholesterol 196 mg. per cent, alkaline phosphatase 1.8 Bodansky units, and acid phosphatase 2.7 King-Armstrong units. The erythrocyte sedimentation rate was 24 mm. in one hour and the Wassermann test was negative. The urinary Sulkowitch test (7) was negative. The 17-ketosteroid excretion was 9.3 mg. in twenty-four hours. The phenol-sulphonthalein excretion was 62 per cent in two hours. The basal metabolic rate was minus 11 per cent. The electrocardiogram was not remarkable except for a P-R interval of 0.22 seconds, and low T waves in leads 1, CR₁ and CR₅.

Roentgenograms of the skull revealed a normal cranial vault with a normal sella turcica and pineal body but extensive bilateral calcification in the parietal and occipital

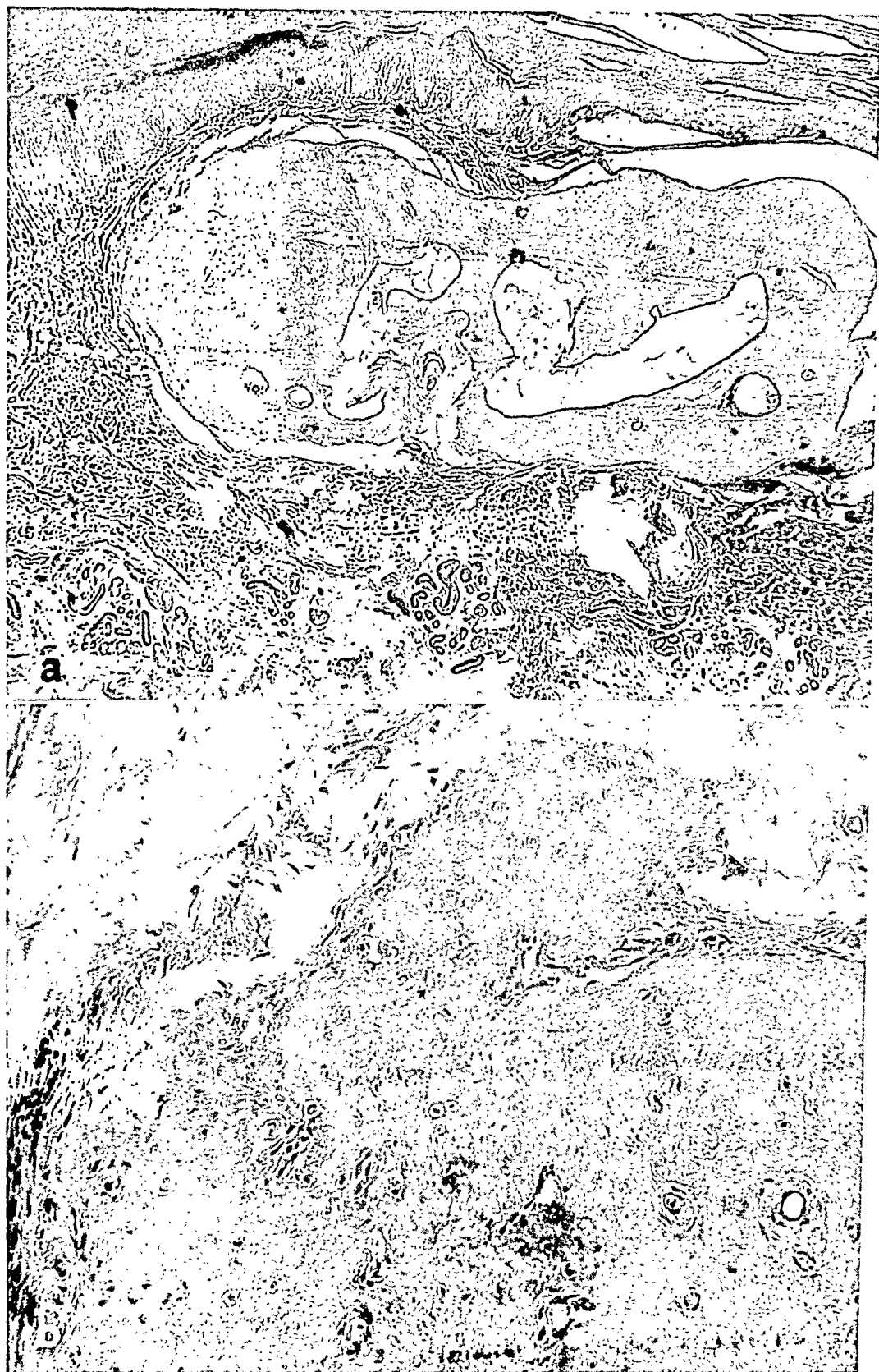


FIG. 6. Photomicrographs of the subcutaneous plaque removed from the left hand.
Note the bony trabeculae, with osteoblastic activity at the periphery.
a) $\times 90$. b) $\times 400$.

regions (Fig. 4). Roentgenograms of the hand, legs, and feet showed extensive calcium deposits in the soft tissues, tendons, and bursae (Fig. 5). An intravenous pyleogram showed normally functioning kidneys without calculi. There was some hypertrophic lipping of the lumbar vertebral bodies.

Two of the subcutaneous plaques on the left hand were removed for study. On dissection they had the consistency of bone. Biochemical assay showed the material to consist chiefly of calcium phosphates of about the same composition as bone. Histologic study revealed bony trabeculae showing osteoblastic activity, with areas of abundant fibrous tissue and scattered cells resembling hematopoietic elements (Figs. 6a and 6b).

An Ellsworth-Howard test (8) was carried out, 2.0 cc. (200 units. U.S.P.) of the parathyroid hormone being given intravenously. The results are shown in Table 1 and are compared with that of a normal control. No phosphorus diuresis occurred in our patient whereas there was a definite phosphorus diuresis in the control with a transitory fall in

TABLE 1

Time	Urinary phosphorus		Urine calcium		Serum inorganic phosphorus		Serum calcium	
	Patient V.S.	Control	Patient V.S.	Control	Patient V.S.	Control	Patient V.S.	Control
	mg./24 hrs.	mg./24 hrs.	mg./24 hrs.	mg./24 hrs.	mg. %	mg. %	mg. %	mg. %
8-9	50.5	14.0	3.7	1.4				
9-10	50.5	24.2	3.9	10.3	1.5	4.1	8.0	10.6
10 a.m.	200 units Parathyroid extract I.V.							
10-11	36	61.5	3.6	2.4	1.6	3.8	8.1	10.9
11-12	29	22.6	2.4	1.0	1.5	4.1	8.4	11.8
12-1	21.7	12.6	<2.0	Trace				
1-2	13.8	39	<2.0	9.0	2.0	4.2	8.2	13.8

the serum inorganic phosphorus. The serum calcium of the patient remained essentially unchanged, whereas that of the control showed a late rise. In our opinion, these results signify that the patient was refractory to the parathyroid hormone.

It was thought that the patient presented the findings of pseudohypoparathyroidism, which had been present since birth, with cataracts and extensive tissue calcification as the late results of a life-long disturbance of calcium and phosphorus metabolism.

The administration of AT-10 (dihydrotachysterol) was begun on April 17, 1948, and the patient was discharged on May 10, 1948, to be followed in the Out-Patient Department. Graphic tabulation of serial calcium and phosphorus determinations during this and subsequent therapy is presented in Figure 7. At first the large dose of 3.0 cc. (3.75 mg.) of AT-10 daily was given. As the serum calcium rose, this was reduced to 1.5 cc. (1.87 mg.) daily, and later to 0.5 cc. (0.625 mg.) daily after calcium chloride, 4.0 Gm. daily, was added. On the latter maintenance schedule the serum calcium was maintained at an approximately normal level. Serum phosphorus had not been abnormally high before treatment but showed some lowering while AT-10 was being given.

development of intracerebral calcification is common to both conditions. The blood in both shows a lowered serum calcium with elevated serum phosphorus and a normal phosphatase level.

Certain definite criteria differentiate the two conditions. All of the reported cases of pseudohypoparathyroidism have shown a characteristic physical appearance (1, 4, 5). The stature is short, with particular shortening of the long bones in relation to the trunk. The face is round with the neck thick and tapering. The hands are stubby and fat with short rounded fingers. The metacarpal bones may be particularly short.

While intracerebral calcifications occur in both conditions, extensive calcification in the subcutaneous tissues of the body is said to be very characteristic of pseudohypoparathyroidism (20).

The final diagnosis of pseudohypoparathyroidism depends on the demonstration of refractoriness to the intravenous administration of parathyroid hormone. Ellsworth and Howard (8) have shown that both the normal individual and the true hypoparathyroid patient respond to a test dose of parathyroid hormone with an immediate and striking phosphorus diuresis followed by a fall in serum phosphorus and later by a rise in serum calcium. The patient with pseudohypoparathyroidism shows no response at all, being refractory to the parathyroid hormone. When this test is performed, previous recent parathyroid hormone therapy must be excluded, as the development of antihormones can produce a negative result. Renal disease which might interfere with phosphorus excretion must likewise be excluded.

The pathogenesis of pseudohypoparathyroidism is entirely unknown. Most of the previously reported cases have been in very young individuals and in our patient the process would seem to have been present since birth. The skeletal alterations suggest that this is a congenital disturbance. The extensive degree of tissue calcification may possibly reflect the life-long duration of the process as compared with the briefer duration of true hypoparathyroidism, a disease acquired later in life.

It is of interest that our patient had convulsive seizures for several years in earlier life but overcame these spontaneously. At no time during our period of observance were the Chvostek and Trousseau signs positive, in spite of very low serum calcium levels. It is suggested that some compensatory adjustment in the nervous system may have taken place, alleviating the excessive irritability usually accompanying hypocalcemia. Likewise, the serum phosphorus in our patient was within normal limits before treatment. Albright (21) postulated that cataract formation and tissue calcification result more from hyperphosphatemia than from hypocalcemia. It is possible that our patient had previously had higher serum phosphorus values which had been reduced by some homeostatic mechanism.

Our patient showed definite evidence of early Parkinsonism. There was

Because of the expense of such dosage of AT-10 and because Sprague (4) and others have shown that vitamin D is as effective as AT-10 in pseudohypoparathyroidism, it was decided to try vitamin D in large doses instead of AT-10. On June 30, 1948, AT-10 was discontinued and vitamin D, 100,000 units daily, was begun. The same dosage of calcium chloride was continued. On this program there was a further rise in the serum calcium with a fall in serum inorganic phosphorus. Because of the marked rise in the serum calcium, the vitamin D was reduced to 50,000 units daily and finally to 50,000 units every other day. The daily dose of 4.0 Gm. of calcium chloride was continued. The comparison of these two therapeutic agents will be further discussed below. On this schedule the serum calcium returned to approximately normal levels.

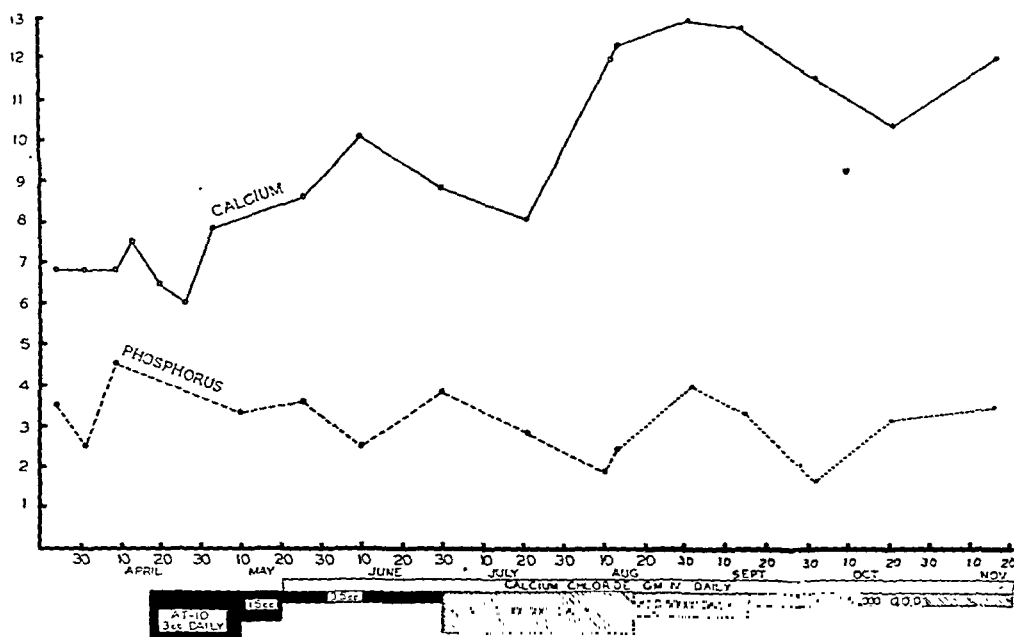


FIG. 7. The effect of dihydrotachysterol (AT-10) and vitamin D on the serum calcium and phosphorus in patient V. S.

For some reason the patient's pains in the back and limbs improved considerably after he began treatment. Presumably his pain was caused by irritation of soft tissues and nerve fibers by the extensive deposits of calcium present. No change in the calcium deposits already present was expected, and none was observed. Nevertheless, the patient considered himself very much improved and was pleased with his treatment. He returned to work as storekeeper and has not missed any time from his job for the past six months.

DISCUSSION

Pseudohypoparathyroidism presents most of the clinical features seen in true hypoparathyroidism (9-19). Tetanic manifestations or convulsions associated with the chronic hypocalcemia are the most outstanding symptoms in both conditions. The tendency to cataract formation and to the

SUMMARY

1. A case of pseudohypoparathyroidism, first diagnosed at the age of 49, is reported.
2. The clinical features of pseudohypoparathyroidism are presented.
3. Very extensive tissue calcification, spontaneous cessation of convulsions, and a serum phosphorus level within normal limits were unusual features in this case. Their possible relationship to the long duration of the disease is discussed.
4. Large doses of vitamin D, with supplementary oral calcium administration, appeared to be a suitable form of treatment.

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no history of any illness resembling encephalitis. Disturbances of cerebral function have been commonly observed in true hypoparathyroidism (13, 22) and their relationship to intracerebral calcification has been discussed (13, 23, 24). The consensus seems to be that the disturbed cerebral function and the calcification both result from brain damage due to metabolic causes. It seems likely that such damage had occurred in the basal ganglia of our patient. No change in the signs of Parkinsonism was observed after treatment.

Histologic study of the calcific fragment removed from the finger of our patient showed a structure remarkably similar to true bone, and what appeared to be true osteoblastic activity. The mechanism of the formation of heterotopic bone is not clear. Duncan (25) states that it may occur in *calcinosis universalis*. Rothstein and Welt (26) cite a case in which a large sequestrum histologically consisting of bone lamellae was removed from *calcinosis* of the anterior abdominal wall. The question arises as to whether fibroblasts of the subcutaneous tissues have taken over osteoblastic function in the presence of altered calcium and phosphorus metabolism.

As to the choice of preparations for treatment, consideration must be given to the mechanism of their action and to the aims of treatment. When tetanic or convulsive manifestations are present, elevation of the serum calcium is obviously the most urgent need. In true hypoparathyroidism this may be accomplished either by AT-10 (11, 14, 16) or large doses of vitamin D (17), together with supplementary oral calcium. If hyperphosphatemia is important in the causation of cataracts and tissue calcification, as Albright believes (21), AT-10 would appear to be preferable because of its greater effect in promoting phosphorus excretion and directly lowering the serum phosphorus (11). Usually a small maintenance dose of AT-10 is sufficient. Where larger doses are necessary, the expense of such treatment may be considerable. In these cases, the simultaneous use of vitamin D with a smaller dose of AT-10 seems reasonable.

In pseudohypoparathyroidism AT-10 is effective, but larger doses are required (1). This is probably due to the fact that these patients are as refractory to the phosphorus-excreting action of AT-10, as they are to the parathyroid hormone. AT-10 is effective then only in so far as it increases calcium absorption. This effect is essentially the same as that of vitamin D. Sprague (4) and his co-workers have shown that in pseudohypoparathyroidism the effects of AT-10 and vitamin D are identical. From our observations with the patient reported here, it appears that vitamin D is as effective as AT-10 and somewhat less expensive. Vitamin D in large doses, combined with supplementary oral calcium, would appear to be the treatment of choice.

CELLULAR INVOLUTION IN THE THYROID GLAND*

SIGNIFICANCE OF HÜRTHLE CELLS IN MYXEDEMA, EXHAUSTION
ATROPHY, HASHIMOTO'S DISEASE AND THE REACTIONS TO
IRRADIATION, THIOURACIL THERAPY AND
SUBTOTAL RESECTION

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ALTHOUGH the eosinophilic cells present in the so-called Hürthle cell tumors probably bear little relation to the interfollicular cells in the thyroids of young dogs described by Hürthle many years ago, prolonged usage has inextricably associated his name with these elements. It has been pointed out that Hürthle cells represent a peculiar type of cellular transformation which occurs in a variety of conditions, but their existence in certain tumors (1) has been much more widely recognized than their presence in non-neoplastic lesions of the thyroid.

A review of the numerous specimens of thyroid received at the Army Institute of Pathology between August 1942 and January 1948 made it clear that the development of Hürthle cells takes place not only in thyroid hyperplasia, in Hashimoto's disease and in exhaustion atrophy but as a consequence of irradiation, administration of thiouracil and partial thyroidectomy. This study was designed to determine the common factors which, operating under apparently widely diverse circumstances, result in the same phenomenon. It is to be hoped that investigation of this peculiar cellular reaction may aid in the formulation of certain biologic concepts which will apply to the other endocrine glands as well as to the thyroid.

Certain epithelial alterations, the most prominent of which is the formation of Hürthle cells, will be referred to under the designation "cellular involution." Cellular involution should be distinguished from follicular involution, that process in which colloid is redeposited while follicular epithelium is reconverted from the active to the resting state.

The classic Hürthle cell is eosinophilic, but variants with a cytoplasm which is foamy or finely stippled with granules are also encountered. Hürthle cells tend to be square rather than columnar and often have a

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The tissue left after subtotal resection for hyperthyroidism exhibited transformation to Hürthle cells in several instances. In some cases the residual tissue assumed the form of a pleomorphic nodular goiter (Plate 2; Fig. 7) in which nodules of Hürthle cells (Plate 2; Fig. 8) were conspicuous. In others (Plate 3; Fig. 11) the transformation was so uniform that the over-all pattern was indistinguishable from that of Hashimoto's disease. In a few instances thyroid parenchyma, composed almost entirely of Hürthle cells, had proliferated so extensively after repeated operations for recurrent exophthalmic goiter that the appearance of a tumor was simulated.

In several cases more than one factor could have been held responsible for the involutionary changes. For example, Figure 9 (Plate 3) shows a focal aggregation of Hürthle cells in the thyroid of a patient who had undergone resection twice and had received a course of thiouracil. This nodule could have resulted from prolonged toxic hyperplasia, from the treatment with thiouracil or from the operations.

The thyroid in Figures 10 and 11 (Plate 3) is from a patient who had had several operations and a course of roentgen therapy. Tissue which had been removed from the thyroid of this patient eight and four years prior to the final operation was available for study. Early involutionary changes were recognized in the earliest specimen, and there was unequivocal formation of Hürthle cells in the tissue removed four years later (Fig. 10). Eight years after the original operation, when the third resection was carried out, examination showed extreme involution and the formation of so many Hürthle cells that the resultant picture (Fig. 11) duplicated exactly the pattern seen in Hashimoto's disease (Fig. 12).

Lymphocytic infiltration was a frequent concomitant of cellular involution. In the study of Hashimoto's disease so much attention has been paid to the lymphoid component that the far more significant epithelial alterations have received due emphasis only relatively recently (2). Transformation to Hürthle cells and desquamation into the follicular lumens was more marked in Hashimoto's disease than in most other types of in-

FIG. 1. (Neg. No. 103291; Acc. No. 96365) Myxedema. Most of the thyroid parenchyma has been replaced by connective tissue. A few nodules of Hürthle cells remain. ($\times 185$)

FIG. 2. (Neg. No. 103281) Exhaustion atrophy. Thyroid of an elderly woman who died of carcinoma of the breast. Dark Hürthle cells form solid nests. Cells exhibiting an intermediate stage of involution are present in the few remaining follicles. ($\times 185$)

FIG. 3. (Neg. No. 103278; Acc. No. 42121) Reaction to irradiation. Numerous Hürthle cells are present in hyperplastic epithelium. ($\times 185$)

FIG. 4. (Neg. No. 103280) Reaction to irradiation. Darkly eosinophilic Hürthle cells are abundant in a gland exhibiting many pleomorphic nodules composed of atypical and bizarre elements. ($\times 185$)

distinct cellular border. Their being closely packed, their size and their shape give a "solid" appearance to areas in which they are abundant. Bizarre, large and distorted nuclei are common; often the nuclei are pyknotic and crenated, with irregular scalloped indentations where the cytoplasmic mass impinges on the nuclear membrane. Follicular conformation is often preserved, but the follicles tend to be small and round and may contain bright eosinophilic colloid material, often of a shade identical with that of the cellular cytoplasm. In advanced stages of involution only scattered nests and cords of polyhedral eosinophilic cells remain in the stromal connective tissue.

In some of the thyroids from patients with myxedema (Plate 1; Fig. 1) no normal parenchymal tissue was left. In the fibrotic glands there were occasional nests of Hürthle cells which lacked a follicular pattern and were accompanied by lymphocytic infiltration. The picture in so-called exhaustion atrophy was comparable, but involution was neither as uniform nor as profound (Plate 1; Fig. 2). It should be pointed out that the time honored term "exhaustion atrophy" indicates that the involutionary nature of the process has been recognized.

Although the reaction of the thyroid to various types of irradiation has been subjected to close study, no typical or characteristic histologic pattern has emerged. In some of the specimens which were studied after irradiation, features of atypical nodular adenomatous goiter were recognizable in addition to many foci of Hürthle cells (Plate 1; Fig. 3 and 4) and other involutionary elements.

Nodules of Hürthle cells were not infrequently seen in diffuse toxic goiter, especially when the hyperthyroidism was of long standing. It was sometimes possible to recognize in hyperplastic thyroid epithelium the early stages of transformation to Hürthle cells, particularly when the alteration was focal. The tall columnar cells broadened, the cytoplasm became clear or eosinophilic and the nuclei exhibited deviations from the normal.

The involutionary changes consequent to the administration of thiouracil are to be compared with those in simple hyperplasia for two reasons: first, because the drug is used for the treatment of toxic hyperplasia, and second, because it itself provokes hyperplasia of thyroid epithelium. In the glands of a few patients treated with thiouracil there was extensive formation of Hürthle cells (Plate 2; Fig. 5) in addition to the peculiar intensification of pre-existing hyperplasia which is often associated with the administration of this drug. The entire thyroid of one patient with hyperthyroidism who was treated with thiouracil consisted of nodules of Hürthle cells (Plate 2; Fig. 6) arranged in papillary conformations so bizarre that a diagnosis of neoplasia had to be considered.

volutionary alteration. In Hashimoto's disease the transition from cellular hyperplasia to cellular involution was evident. The belief expressed by Hellwig that Hürthle cells are "... young proliferating rather than degenerated and atrophic ..." elements is based in part on his recognition that in the thyroid of Hashimoto's disease there is an intimate relation between Hürthle cells and hyperplastic elements. That the involution of hyperplastic epithelium is at times paralleled by functional alteration is evidenced by the old clinical observation that Hashimoto's disease and hypothyroidism may be sequelae of hyperthyroidism.

Certain types of suppressive treatment increased the tendency to cellular involution, which is a consequence of prolonged stimulation. For example, Hürthle cells were often seen in simple hyperplasia; irradiation or treatment with thiouracil accelerated their formation. Similarly, in some instances in this series, continued proliferation of thyroid tissue despite multiple resections, resulted in cellular involution of the most extreme type.

Cellular involution is caused by prolonged hyperplasia without follicular involution (Fig. 13). It must be emphasized that such morphologically demonstrable hyperplasia is not necessarily associated with hyperfunction in the sense of excessive secretion. Deficiency of iodine or the administration of thiouracil, for example, result in hypofunction with concomitant cellular hyperplasia. Compensatory hyperplasia of thyroid epithelium ensues when there is failure of normal secretion. Either simple hyperplasia over a prolonged period or exaggerated hyperplasia, such as that caused by thiouracil, over a short period can result in cellular involution.

SUMMARY

By involution of the thyroid ("follicular involution") is meant that process in which follicular colloid is redeposited and follicular epithelium

FIG. 5. (Neg. No. 103335; Acc. No. 195181) Reaction to thiouracil. Focal transformation to Hürthle cells of hyperplastic thyroid epithelium. This patient had received thiouracil until ten days before resection was carried out. There were also many foci of atypical hyperplasia and pseudoneoplastic papillary conformations. ($\times 185$)

FIG. 6. (Neg. No. 103372; Acc. No. 168236) Reaction to thiouracil. Papillary and adenoid nodules composed almost exclusively of Hürthle cells in the thyroid of a man treated with thiouracil for exophthalmic goiter. ($\times 23$)

FIG. 7. (Neg. No. 103382; Acc. No. 198384) Reaction to partial thyroidectomy. Many nodules composed of Hürthle cells in a recurrent nodular goiter. The first operation had been performed eight years before (*see* Fig. 8). ($\times 11$)

FIG. 8. (Neg. No. 103337; Acc. No. 198384) Reaction to partial thyroidectomy. High power view of one of the nodules illustrated in Figure 7. It is composed entirely of dark Hürthle cells. ($\times 185$)



Fig. 1

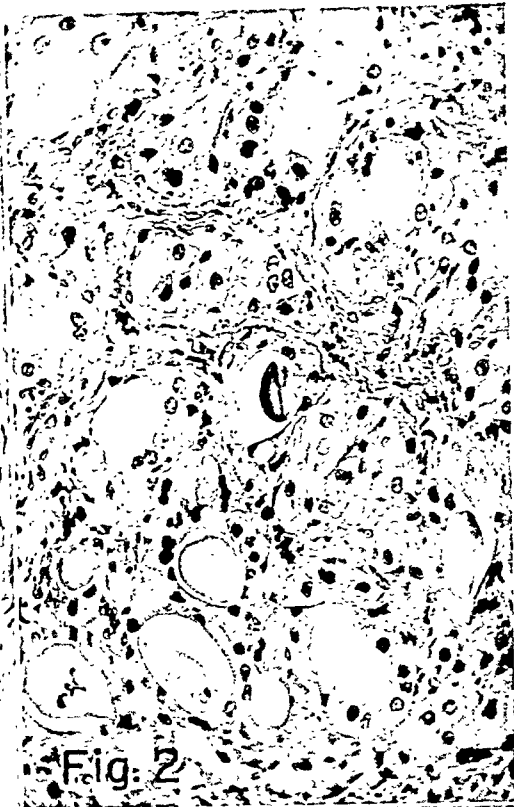


Fig. 2

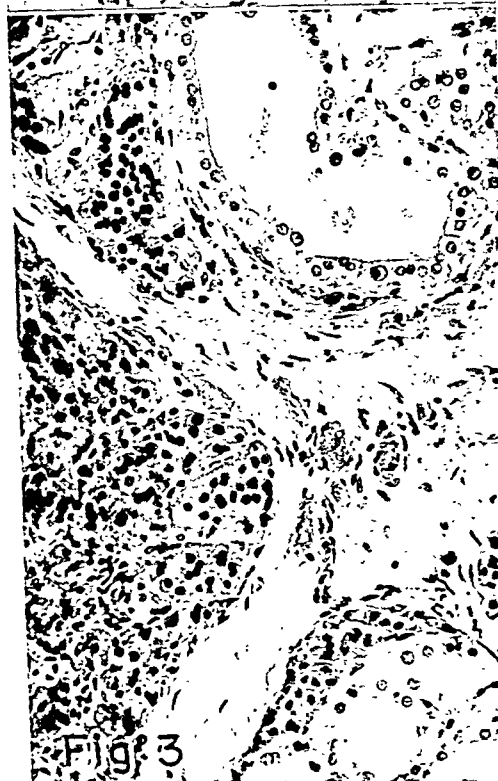


Fig. 3

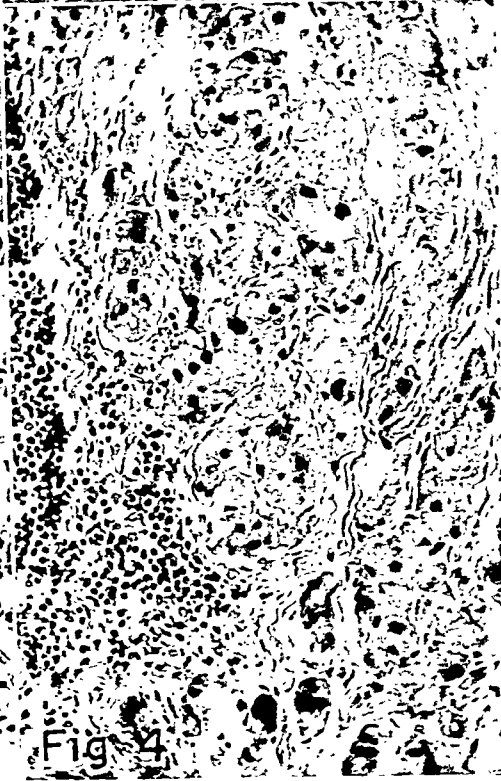


Fig. 4

PROLONGED HYPERPLASIA, CELLULAR INVOLUTION (HASHIMOTO'S DISEASE)

HYPERPLASIA

INVOLUTION
(follicular)

NORMAL RANGE OF CYCLIC VARIATION

IRREGULAR INVOLUTION (follicular)
HYPERINVOLUTION (follicular)

(NODULAR, ADENOMATOUS GOITER)
(COLLOID GOITER)

FIG. 13. Diagram indicating the difference in genesis of follicular and cellular forms of involution. The normal thyroid gland may undergo a recognizable degree of cyclic hyperplasia and follicular involution without resultant significant permanent structural alteration. Repeated cycles of excessive hyperplasia and follicular involution result in nodularity because proliferation and regression in different portions of the gland are not synchronized (3). Persistent follicular hyperinvolution leads to the development of colloid goiter.

returns to the flattened resting state after a period of hyperplasia. This phenomenon contrasts with certain other epithelial alterations which take place under a variety of conditions and might well be termed "cellular involution." The most prominent of these changes is the formation of the eosinophilic elements commonly called Hürthle cells in lesions of the thyroid generally regarded as regressive. Recognizing that cellular involution is common to a number of diseases may aid in the understanding of the pathologic physiology of the thyroid gland.

Numerous examples of cellular involution in the thyroid have been studied during the past five years at the Army Institute of Pathology. The conditions in which the phenomenon was observed included Hashimoto's disease, myxedema, "exhaustion" atrophy and exophthalmic goiter. It is of particular interest that identical alterations were observed consequent to roentgen therapy, treatment with thiouracil and subtotal resection. The occurrence of cellular involution can be taken as an indication that the

FIG. 9. (Neg. No. 103375; Acc. No. 184612) Reaction to partial thyroidectomy and thiouracil. Nodule of Hürthle cells in a gland which had twice been subjected to subtotal resection. The patient had also received thiouracil. ($\times 55$)

FIG. 10. (Neg. No. 103288; Acc. No. 86772) Reaction to irradiation and partial thyroidectomy. Focal development of Hürthle cells in a hyperplastic gland resected for the second time four years after irradiation and partial thyroidectomy, for exophthalmic goiter. Material removed at a third operation four years later revealed more diffuse and advanced cellular involution (see Fig. 11). ($\times 185$)

FIG. 11. (Neg. No. 103288A; Acc. No. 86772) Reaction to irradiation and partial thyroidectomy. This pattern was encountered in material removed at the third operation on the patient mentioned in the legend of Figure 10. There had been considerable regrowth of thyroid tissue. The small follicles composed of Hürthle cells and the lymphoid infiltration are identical with those seen in Hashimoto's disease (see Fig. 12). ($\times 185$)

FIG. 12. (Neg. No. 103294; Acc. No. 185263) Hashimoto's disease. Diffuse formation of Hürthle cells and lymphoid infiltration. Compare with Figure 11. ($\times 185$)



Fig. 6

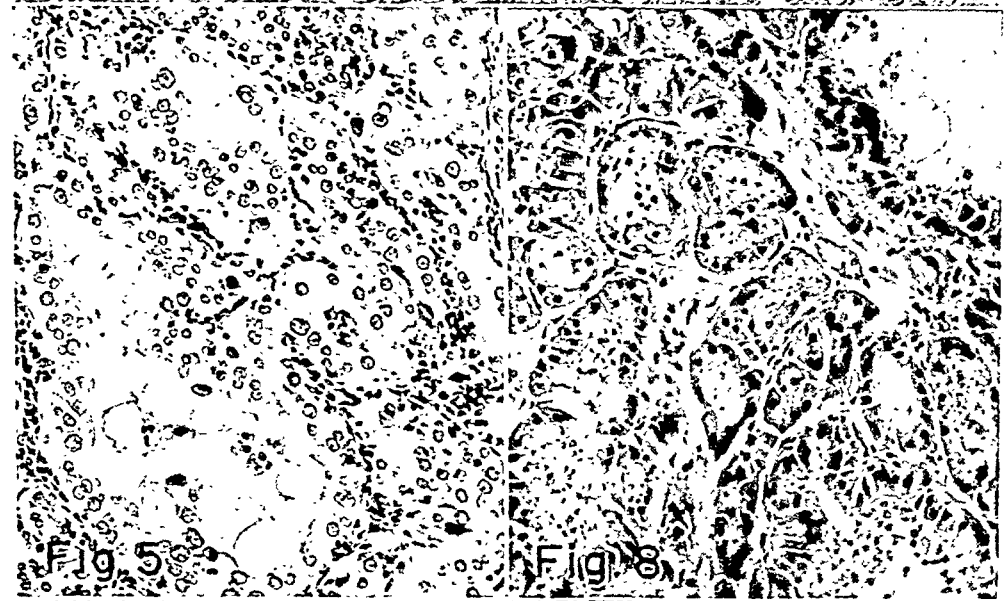


Fig. 5

Fig. 8



Fig. 7

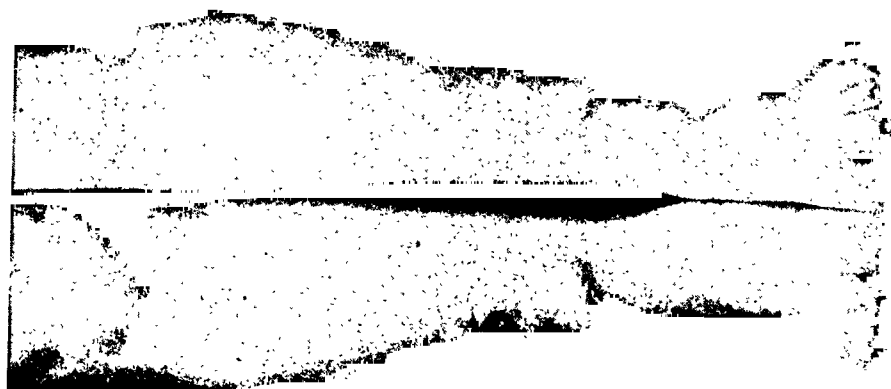


Fig. 1. Showing the cutaneous lesions in case 1. The scar of biopsy is still visible in the right leg.

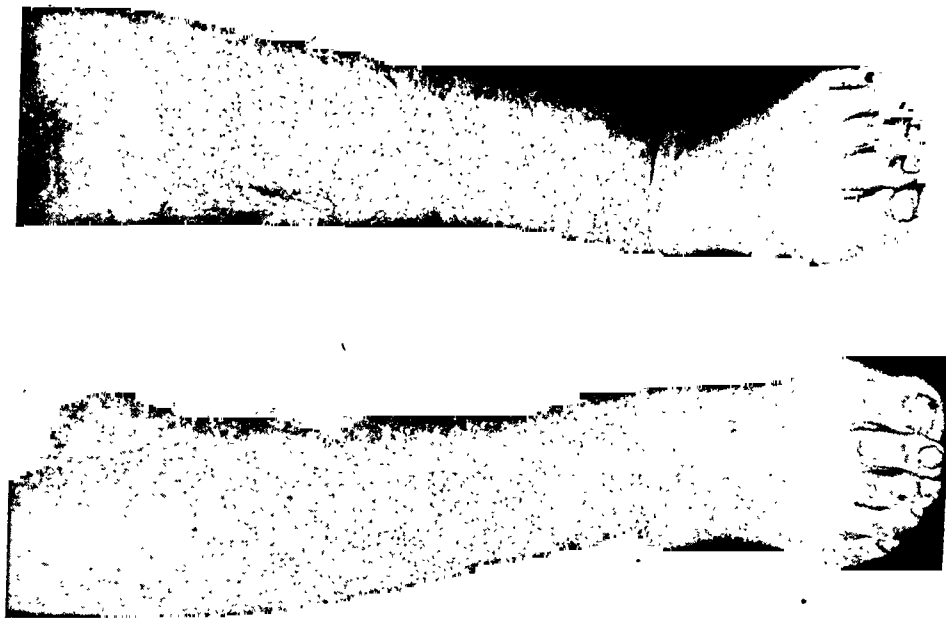


Fig. 2. Showing the cutaneous lesions in case 2.



Fig. 9

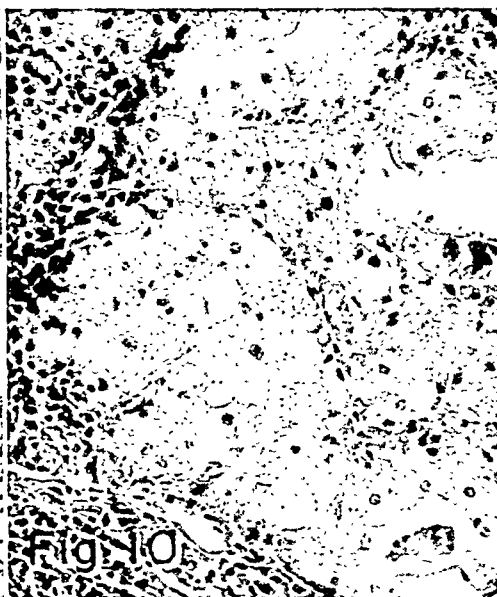


Fig. 10

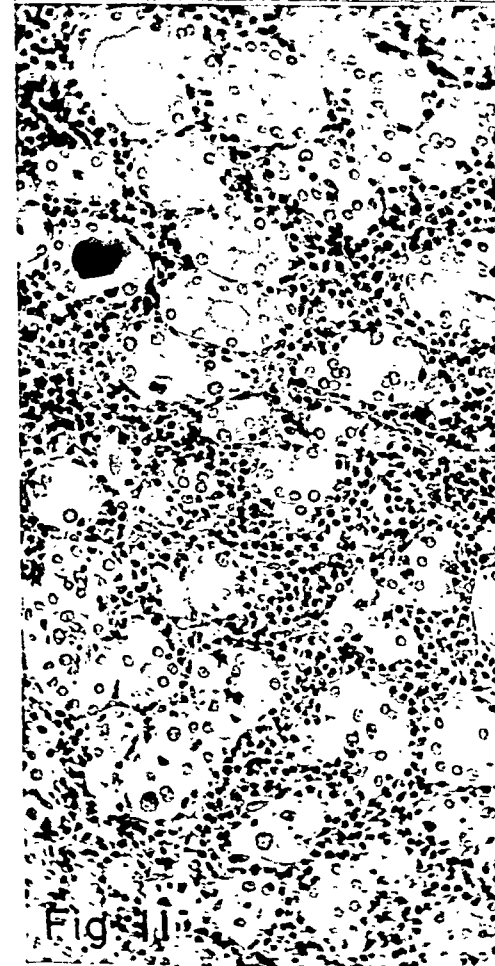


Fig. 11

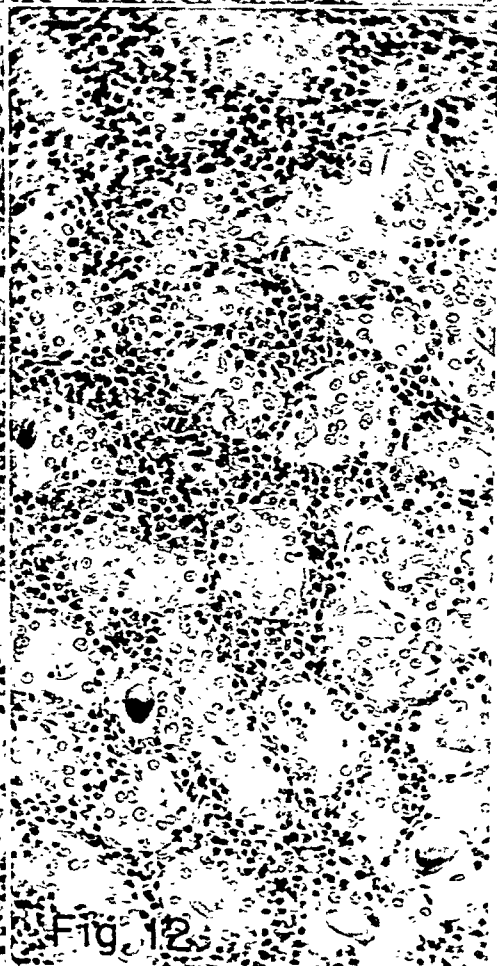


Fig. 12



Fig. 3. Case 1. Note marked exophthalmos and edema of eyelids.

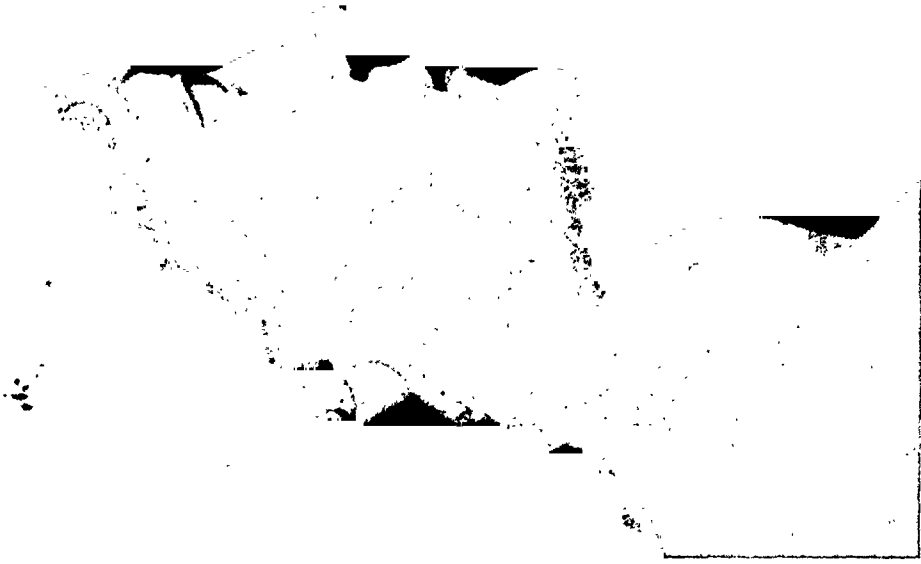


Fig. 4. Case 1. Lateral view of the exophthalmos and goiter.

CASE REPORTS

Case 1. Carmen J. was a 20-year-old girl from Lérida. Family and past history revealed nothing pertinent.

In December 1945 she showed nervousness, insomnia and asthenia. In a few weeks she lost 8 Kg. in weight. In February 1946 the presence of a diffuse goiter was observed and at the same time she complained of palpitation and diarrhea. The condition was diagnosed as hyperthyroidism and she was treated with homeopathic methods for three consecutive months, without improvement. In October of the same year, bilateral exophthalmos appeared and soon afterwards a striking improvement commenced. In September 1947 two indurated patches developed in both pretibial regions; they were clearly defined, of cyanotic appearance and slightly pruriginous. This cutaneous alteration developed during the course of about two weeks and then remained stationary.

On August 26, 1948, the basal metabolic rate was plus 32 per cent. The attending physician made a diagnosis of Graves-Basedow disease and prescribed 0.30 Gm. daily of methylthiouracil. This treatment was continued until October 28, when the patient was seen by us. With the antithyroid medication her nervousness, palpitation, fatigue and diarrhea improved considerably but the goiter, the exophthalmos and the cutaneous lesions were intensified.

Physical examination. Weight 63 Kg.; height 1.60 M. There was marked bilateral exophthalmos (Figs. 3 and 4) with slight edema of the eyelids (exophthalmometry RE=22 mm., LE=23 mm.). Gräfe, Möbius, Stellwag and Dalrymple signs were present. There was a diffuse soft goiter with a vascular systolic thrill all over. The pulse was 160 and the blood pressure, 140/75 mm. Hg. The tendon reflexes were exaggerated.

Occupying the union of the medium third with the inferior third of both legs and situated on the antero-lateral surfaces, there were large patches, 15 cm. high by 10 cm. wide on the right extremity, and 20 cm. high by 12 cm. wide on the left. These patches were elevated by about 2 cm. from the surrounding skin. They were of a reddish-violet color, cyanotic, of a hard consistency, non-pitting, and perfectly delineated. Over their surface there was observed an adherent discrete desquamation, which when lightly scraped showed dilated follicles and a hypertrichosis. The hairs were not very numerous, were irregularly distributed, and were not found on the skin of the rest of the extremity (Fig. 1).

Both patches resembled the enormous blisters of Quincke's edema, from which, however, they were distinguished by their slow development, purple color, firmer consistency, the fine overlying desquamation and the hypertrichosis.

Biopsy of skin. (October 29, 1948). The epidermis conserved its normal structure, but the interpapillary prolongations were very strongly marked. The granular layer was hypertrophic and corresponded to the manifest hyperkeratosis throughout the skin, which was exaggerated still more at the level of the follicle orifices. The elective stains for melanin showed an increase of this element and its retention by the dermic chromatophorous cells.

In the papillary layer the elastic fibers were abnormal. The elastic fibrils had almost completely disappeared and the collagenous fibers had lost their fibrillar appearance. Some capillaries were observed surrounded by small cellular infiltrations that were chiefly lymphocytes.

The most marked lesions were seen in the corium. The fibrous layer of the skin was found to be completely divided by a clear edematous zone into two layers (Fig. 5), one superficial and the other deep, in which the pathology was practically the same. The

already described at other levels of the skin. Also around some vessels there were small lymphomonocytic infiltrations, more discrete than in the rest of the corium. In this edematous zone were found the pilosebaceous follicles. The fibers of the pili erector muscles appeared swollen and very refractive, whereas the sudoriparous glands were unaltered.

The hypodermis was normal, except for a panvascularitis affecting only the small arteries and veins feeding the skin.

Laboratory findings. The basal metabolic rate (October 29, 1948) was plus 12 per cent, blood cholesterol 145 mg. per cent, creatine retention 60 per cent, and urinary excretion of 17-ketosteroids, 10.7 mg. in twenty-four hours.

Thyrotropic hormone in the urine assayed 6.25 Junkmann-Schoeller units. Extraction was done by the Emerson and Cutting method (4); titration was done on the 24-hour-old chick by the Rawson and Salter microhistometric method (5).

Hematologic examination. Plasma and serum proteins and blood potassium, sodium, calcium and glucose levels were normal. The red and white blood cell counts were normal but there was a lymphomonocytosis.

Case 2. Maria de los Angeles T. was a single woman from Barcelona, aged 42 years. Family and past history were not pertinent.

The first signs of her illness began in September 1947 after an intense psychic trauma. She complained of heart pain, giddiness and palpitation. She was treated with digitalis during several months without relief. After January 1948, menstruation became less frequent and irregular. In March of the same year she observed bilateral exophthalmos which became progressively more intense.

On April 12, 1948, she was visited by one of us. She presented a very intense exophthalmos (exophthalmometry RE = 24 mm. LE = 23 mm.) with epiphora, palpebral edema and Gräfe, Möbius and Stellwag signs. Her thyroid gland was palpable. The heart rate was 130, with regular rhythm and the blood pressure was 150/70 mm. Hg.

Her basal metabolic rate was plus 44 per cent, and blood cholesterol 110 mg. per cent. Blood cell counts were normal, but there was a lymphocytosis.

With the diagnosis of Graves-Basedow disease, on April 13 treatment was started with 0.50 Gm. of thiouracil daily. On April 26 she was much better, the pulse having decreased to 90 and the metabolic rate to plus 26 per cent. The dose of thiouracil was reduced to 0.30 Gm. daily.

The initial improvement continued slowly and then the patient began to take thiouracil irregularly without any medical control.

On July 10 we saw the patient again with an obvious recurrence of thyrotoxicosis. Since the last fortnight in June she had noticed in both inferior extremities symmetrical cutaneous swellings distributed uniformly over the antero-lateral surfaces of the legs, commencing 3 cm. below the patella and ending 2 cm. above the metatarsophalangeal articulations (Fig. 2).

These swellings were solid, uniform, and did not pit on pressure. They were distributed in the form of a hemicylinder of which the continuity was broken at the level of the ankle by a deep groove shown in Figure 2. The color was a uniform reddish-purple. The cutaneous surface was covered by small funnel-like depressions corresponding to the pilosebaceous orifices, the abundance and uniformity of which gave to these patches the appearance of orange-skin.

On the cutaneous surface there was a fine adherent desquamation and an abundance of hairs, coarse, long and black, situated on the internal surface of both legs. The cutane-

collagenous network was dense but the appearance and coloring of the fibers tended to hyalinization. In certain zones of the microscopic preparation the interstitial lymphatic spaces were expanded and the fibroblasts showed ramification; this edema was only local and did not spread to the network or separate it. The elastic fibers, however, appeared fragmented, straight or in moniliform grains; some were well stained, whereas others had less affinity for the orcein and seemed about to disintegrate. From time to time there were visible at this level some perivascular infiltrations of mononuclear and lymphocytic cells. In the middle part of the corium the edema completely dominated the



FIG. 5



FIG. 6

FIG. 5. Case 1. The edematous zone which divides the corium into two layers is clearly distinguishable. This lesion appears characteristic of this disorder. Note mild hyperkeratosis of the epidermis and normal hypodermis (stain by the Masson's trichromic method).

FIG. 6. Case 1. Detail of the alterations of the elastic fibers in the deeper layer of the corium (stain by the orcein-hematin method).

picture and was so great that the space appeared almost empty (Fig. 6). partitioned only by an amorphous fibrillar wide reticulum which did not stain with thionin, mucicarmin or the acid and basic anilines. Scattered irregularly near this zone, there were a few bits of hyalinized collagenous substance of irregular outline or forming balls, which appeared thicker around some blood vessels which were sectioned at this level. The rare elastic fibers were distributed irregularly and presented more definitely the alterations

80 per cent (Goldner (15)), but in others it appeared in conjunction with a remission of the disease and with metabolic rates near the normal figure (cases of Samek (10), Urbach (13), Cohen (3)) or after thyroidectomy with marked hypometabolism (O'Leary (16), Netherton and Mulvey (17)). In the last type of case the administration of desiccated thyroid produces a rapid disappearance of the hypothyroid signs but the patches of circumscribed myxedema persist unmodified. In some patients the "circumscribed myxedema" develops either before or with the first manifestations of hyperthyroidism (O'Leary (7), Dowling (18)), whereas in other cases, such as our first patient, it appears when the hyperthyroidism has had a long period of evolution. The possibility of a tired thyroid gland might explain the last, but not the first situation.

The "thyrotoxic myxedema" in some cases starts with the formation of a soft diffuse edema, varying in intensity from one day to another and changing finally to the characteristic pretibial patches (O'Leary (16)). In other cases the lesions develop slowly and are delimited from the very first. Nodules have also been observed. These nodules are separate (Doessekker (19), Freudenthal and Brunauer (20)) but by confluence they reproduce the characteristic patches of the lesion (Dowling (18)).

The course of this cutaneous lesion is essentially chronic. In the published cases different hormonal and dermatologic treatments have been tested with practically negative results. Iodine, thyroidectomy and anti-thyroid drugs, desiccated thyroid and thyroxin, have been employed and shown to be useless or even capable of aggravating the process.

The histopathologic pattern of the cutaneous lesions also needs interpretation. According to the greater number of authors (*e.g.*, Pillsbury and Stokes (8), Netherton and Mulvey (17), Carol (11), Cohen (3)) the histologic picture is characteristic of hypothyroid myxedema. Dowling (18) and McLeod (21) speak of a mucinic degeneration of the corium secondary to a basophilic degeneration of the collagen and elastin, and Netherton and Mulvey believe that the lesion is completely different from genuine myxedema. They consider the condition to be a "mucoid degeneration of the skin" associated with hyperthyroidism. However, in our first observation some of the histochemical reactions for mucin were completely negative.

The conservation of the structure and the normal disposition of the papillae eliminates the possibility that these cutaneous lesions are due to a dermic edematous or other infiltration, which no doubt would have distended the epithelium, eliminating or at least decreasing the papillary festoon which was very prominent in our case. On the other hand, the situation of the lesion in the middle zone of the corium gives to the histologic picture a very specific topographic character and if we furthermore con-

ous alteration did not produce any discomfort, except a slight sensation of heaviness and tension.

The basal metabolic rate (July 10) was plus 34 per cent. Blood cholesterol was 125 mg. per cent. No permission for biopsy was obtained.

COMMENT

The cutaneous lesions observed in our 2 patients coincide completely with the published descriptions of "myxedema circumscriptum thyrotoxicum." Both patients presented typical Graves-Basedow disease; in the first patient cutaneous infiltration patches appeared during a phase of spontaneous partial remission of the thyrotoxicosis and coincided with the development of the exophthalmos; whereas in the second patient the "circumscribed myxedema" appeared at the beginning of a recurrence of the thyrotoxicosis after treatment with thiouracil

The pathogenesis of this disorder is a problem not yet solved. According to Sabrazes (6) these patches result from vasomotor alterations, causing blood and lymph stasis and chronic edema (*trophoedème basedowien*). They are capable of resolving spontaneously or developing gradually to scleroderma.

O'Leary (7), Pillsbury and Stokes (8), and Ingram (9) believe that the lesions can be produced by chronic vascular stasis and cardiac failure secondary to the thyrotoxicosis. Traumatism has been considered as a possible causal factor of localization (Samek (10)). The action of cold could be one of the determining factors, according to Samek (10) and Carol (11), who point out the similarities of these alterations with erythrocyanosis crurum.

These hypotheses are not applicable in our cases nor in many others published by different authors in which the indurated patches appeared in mild thyrotoxicosis and even after the thyroid extirpation, without antecedent cardiac failure, traumatism, or the action of low temperatures.

There have been many endocrine hypotheses to explain the origin of these lesions. Richter (12) speaks of a state of thyroid exhaustion and considers that the disorder is produced by a functional alteration of antagonistic endocrine glands. Carol (11) believes that there exists in the legs a local hypothyroid state resembling the vascular disorders caused by cold. Urbach (13) and Wiener (14) think that the skin in the affected region has become incapable of using the thyroid hormone (either in normal quantities or in abundance), resulting in the local myxedematous alterations.

The "circumscribed myxedema" has been observed only in individuals who have or have had exophthalmic goiter. In some cases the patient showed definite thyrotoxicosis with a basal metabolic rate as high as plus

edema" is accentuated even more if we consider the results of the different therapeutic methods which have been tested for each. We have already indicated that the exophthalmos is almost always accentuated after thyroid extirpation and may develop into dreaded malignant exophthalmos; "circumscribed myxedema" appears almost always in the remission phase of the disease and in many cases it only manifests itself after thyroidectomy. The antithyroid compounds such as thiouracil, possibly by their inhibitory action on the production of the thyroid hormone, in our patients produced an increase of exophthalmos and pretibial edema. Treatment aimed at inhibiting the anterior lobe of the pituitary gland (*e.g.*, estrogens, radiotherapy) gave good results in some cases of malignant exophthalmos studied by Friedgood (36), Brian (40), Martens (41) and Klotz (42). We therefore decided to test these procedures also in our cases of "thyrotoxic myxedema."

In the first of our patients we omitted the methylthiouracil and initiated treatment with 2 mg. daily of estradiol benzoate. After fifteen days the pulse rate had dropped from 160 to 80, the tremor of the fingers had disappeared and the weight had increased by 1 kilogram. From that date, the cutaneous patches gradually lost their cyanotic appearance, decreased in height and showed a tendency towards the formation of isolated nodules. The basal metabolic rate dropped from plus 12 to minus 18 per cent. After a month of treatment, a subtotal thyroidectomy was performed by Dr. Piulachs. After the operation the patient continued to take 2 mg. daily of estradiol benzoate in courses of treatment of three weeks' duration followed by a premenstrual interval of therapeutic rest. One month after the operation the metabolism was maintained at minus 17 per cent, the cutaneous patches had been considerably reduced, and the exophthalmos was very much less intense; urinary excretion of thyrotropic factor was not demonstrable.

In the second case the thiouracil was also omitted and treatment was begun with 0.5 mg. of diethylstilbestrol and 20 drops of Lugol's solution daily. The patient progressively improved and after seventeen days the basal metabolic rate dropped from plus 34 per cent to plus 12 per cent. The cutaneous infiltration, however, remained uninfluenced. To the prescribed treatment, 2 mg. of estradiol benzoate was added daily during intermenstrual cycles. After three months we again saw the patient. She had a small pretibial infiltration on the left leg, her exophthalmos had almost completely disappeared and she was free from hyperthyroid symptoms. Her basal metabolic rate was minus 7 per cent.

The action of thyrotropic hormone provides an explanation of the origin of the cutaneous infiltration patches which are observed in some patients with Graves-Basedow disease. Their elective localization on the pretibial

sider the alteration of the collagen and elastic tissue which accompanies it, this infiltration could be interpreted as secondary to the alteration in elastic tissue produced by the endocrine disorder.

The presence of edematous alterations of the skin in patients with primary thyrotoxicosis is observed with relative frequency. The edema can affect the face, the extremities and specially the eyelids; it is transient and some times considered to be of angioneurotic type. It has no relation whatsoever to the intensity of the thyrotoxicosis. The exophthalmos, both that observed in Graves-Basedow disease and that which can be produced in laboratory animals by the administration of thyrotropic hormone, is due also to edema of the intra-orbital structures (Naffziger (22, 23), Naffziger and Jones (24), Jones and Naffziger (25), Aird (26), Smelser (27)); and, as happens in the other forms of edema and with the circumscribed myxedema, it is neither a constant sign of hyperthyroidism nor does it follow the course of the disease. In some cases the exophthalmos appears before any other thyrotoxic symptom (Klotz (28)); in others it manifests itself only in remissions or after the thyroid extirpation (Ruedemann (29)). In general, it increases after operation (Soley (30), Dobyns (31), and Dobyns and Haines (32)).

There exists then a series of analogies between the "circumscribed myxedema" and the exophthalmos. Exophthalmos is not due primarily to thyroid hyperfunction, as Gley (33) was able to produce it in the rabbit by means of thyroid extirpation and Friedgood (34, 35, 36) provoked it in the intact or thyroidectomized guinea pig by injection of pituitary extracts rich in thyrotropic hormone. Clinical and experimental studies have shown that the exophthalmos is a sign of hyperfunction of the anterior pituitary lobe produced through the thyrotropic hormone; or, as Friedgood (36) suggests, by mediation of an exophthalmotropic factor intimately related with this hormone.

"Circumscribed myxedema" is observed only in patients with intense exophthalmos and Cohen (3) refers to observations in which the ocular protrusion was so great that it should be classified as malignant exophthalmos. In such cases, in which the retrobulbar edema and the aqueous infiltration of the eyeball dominate the clinical picture, it is possible to demonstrate the urinary excretion of large quantities of thyrotropic factor, whereas in the hyperthyroidism not accompanied by exophthalmos and in normal individuals the excretion is very low or nonexistent (Rawson and Starr (37), Hertz, Williams and Means (38), Klotz (28, 39)). In our 2 patients there was a severe exophthalmos and in the first one we detected the excretion of a high titer of thyrotropic factor.

The parallelism between the exophthalmos and the "thyrotoxic myx-

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region of both legs possibly is due to the particular conditions under which the blood and lymphatic circulation is effected in this region.

SUMMARY

Two cases of "myxedema thyrotoxicum circumscriptum" are described. In one of the patients the lesions appeared after two years of Graves-Basedow disease; in the second, they developed during irregular treatment with thiouracil. In the first case the basal metabolic rate was plus 12 per cent; in the second, plus 34 per cent. Both patients were cured by the administration of estrogen in large doses.

The cutaneous biopsy showed in one case the presence of an edematous zone which divided the corium into two layers. This histologic lesion, with its specific topography and structure, seems to be characteristic of this disorder.

Circumscribed myxedema is a cutaneous lesion which, like malignant exophthalmos, is apparently caused by high production of pituitary thyrotropic hormone.

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tions. Animal protein or some other substance not removed from the pancreas in extraction may be present in the commercial insulin. Patients with positive reactions to skin tests with insulin obtained from various animal sources as well as with crystalline insulin have shown negative skin reactions with beef, pork, sheep, and horse proteins. These patients apparently manifest a true hypersensitivity to insulin which is not based upon a species-specific factor (6). On the other hand, it is a common clinical observation that patients who react unfavorably to insulin from one source may be able to tolerate insulin derived from another animal. Cohen and Simon (7) reported a case in which cutaneous tests definitely established this fact, indicating that the hypersensitivity was due to the animal protein source of the insulin and not to the pure insulin principle itself.

Insulin modifiers such as protamine have similarly been implicated as causative factors in allergic reactions to insulin. Cohen and Simon (7) state that certain individuals develop local reactions due to the protamine content of the preparation. However, Kern and Langner (8) performed intracutaneous tests with 0.02 cc. of protamine (salmine) solution on 100 control subjects and 104 diabetic persons who were receiving protamine zinc insulin (PZI). No positive reactions were obtained. All reports seem to indicate that positive cutaneous reactions to protamine insulin are always associated with reactions to other insulins such as beef, pork, and crystalline insulin, and not to protamine itself (6). Still other factors have been implicated such as the alcohol in which the syringe is kept, the preservative in the insulin, and the denaturing materials.

There are three types of reactions to insulin: 1) mild local reactions at the site of injection, 2) severe local reactions, and 3) general reactions. The mild local reactions at the site of injection usually consist of erythema or slight local induration and are probably nonimmunologic in nature and of little clinical significance. Severe local reactions usually consist of erythema and local edema, accompanied by pruritus. Such reactions may be immediate or delayed from six hours to several days after the injection. General reactions consist of generalized urticaria frequently associated with angioneurotic edema and with symptoms of circulatory failure, bronchial asthma, and gastro-intestinal symptoms.

The incidence of allergic reactions to insulin varies widely in the reports of different authors, apparently depending upon whether all local reactions as well as general reactions are reported. Hence such wide variations as Grafe's (9) 0.15 per cent, Collens, Lerner and Fialka's (10) 7.3 per cent, and Allan and Scherer's (11) 11.7 per cent have been reported. Allan and Scherer estimate that of persons hypersensitive to insulin, 98 to 99 per cent will show a local reaction and only 1 to 2 per cent a general reaction.

GENERALIZED INSULIN ALLERGY

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INSULIN allergy is the term used to denote the specifically altered, acquired antigenic response to insulin protein in contradistinction to insulin sensitivity, which denotes an altered physiologic response to the hormone.

To illustrate this differentiation, Lowell (1), in extensive immunologic studies, presents evidence indicating the presence of two antibodies for crystalline insulin in one patient who was both allergic and resistant to crystalline insulin. He demonstrated the presence of a heat-labile allergic antibody which was capable of passively transferring skin sensitivity and an insulin-neutralizing antibody which was heat-stable and capable of destroying the physiologic effect of crystalline insulin.

Various workers (2 a, b, c, d) have definitely established that crystalline insulin is a complete and soluble protein and, upon examining the insulins derived from the pancreases of various mammals (ox, sheep, hog) and fish, have found them to be similar in microscopic appearance, isoelectric point, solubility, and in carbon, hydrogen, nitrogen and sulphur content.

A considerable number of animal and human experiments have demonstrated the antigenic activity of insulin (3). Some authors (4 a, b, c) attempted to demonstrate the immunologic identity of the insulins derived from the pancreases of human beings, cattle, hogs, sheep, bison, and dogs. The results indicate that insulin lacked organ and/or species-specific factors. However, Lewis (5) in 1937, employing the Dale-Schultz uterine-strip technique, sensitized guinea pigs with beef or pork insulin and tested uterine strips with homologous or heterologous insulin and with beef pancreas and serum. He concluded that beef and pork insulin are closely related and have antigenic activity in common but are not completely identical, and he believes that there is a residuum of specificity in insulin which is inherent to the species but which differs from the other proteins of the same species.

Other factors have been implicated in the production of the allergic reac-

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Case 2: DJG, a 24-year-old white bus driver, was admitted to Hines Hospital on December 29, 1947, complaining of tender gums, progressive weakness, and weight loss of four weeks' duration. Prior to admission, he had been given a penicillin solution as a mouth wash by his dentist and oral medication by his physician without relief of symptoms. On admission his tonsils were enlarged, the pharynx was injected, and numerous small white ulcerations were seen on the buccal mucosa and gingival margins. A smear taken from the ulcerations showed *Spirilla* and fusiform bacilli. A single urine specimen showed 4 plus sugar and acetone. The initial blood sugar was 250 mg. per cent and the CO₂ combining power was 28 vols. per cent. Twenty-four hour quantitative urinalysis showed 75 Gm. of sugar in 3000 cc. of urine. His diabetic acidosis was readily controlled with intravenous fluids, crystalline insulin, and protamine zinc insulin. After five days of insulin therapy, he awoke on the morning of January 4, 1948, with extreme puffiness of the eyelids and lips and a generalized urticaria with intense pruritus. The protamine zinc insulin was discontinued and maintenance therapy on crystalline zinc insulin was attempted. However, his allergic manifestations grew worse despite use of antihistaminic drugs. Intradermal tests with beef and pork extract and protamine were negative but a 4 plus reaction was obtained with crystalline zinc insulin. The intracutaneous tests were repeated using amorphous regular insulin, crystalline zinc insulin, PZI, and globin insulin. There was a positive skin reaction to all these preparations.

On January 13 the patient was desensitized to PZI according to the rapid method described by Bayer (12), starting with 1/1000 unit intradermally and doubling the dose ever fifteen minutes until 1 unit was reached, followed by subcutaneous injection to the dose required for control of symptoms. Then daily injections of PZI were given. There was a mild generalized urticaria on January 15, but no symptoms at all after this. The patient was discharged on January 20, taking 24 units of PZI daily on a 2600 calorie diet.

He was readmitted on March 3, 1948, complaining of polyuria, polydipsia, polyphagia, weakness, fatigability, and weight loss. He stated that since release from the hospital, his urine tests were negative for sugar and he had gradually decreased his insulin dosage, finally maintaining himself on diet alone and discontinuing insulin altogether. A few weeks later, glycosuria again appeared but on resuming insulin severe urticaria recurred and he was unable to take his insulin. The urine was strongly positive for sugar and acetone, blood sugar was 420 mg. per cent and CO₂ combining power was 44 vol. per cent. He was again desensitized to PZI according to the method of Bayer and concurrently was given pyribenzamine, 100 mg. four times. On this regime, occasional urticaria was noted for the first few days, which subsequently entirely disappeared. Protamine zinc insulin was started; subsequently crystalline zinc insulin was given twice daily as supplemental dosage, and he finally was given a 2:1 insulin mixture, which adequately controlled his diabetes. He was advised in the future to take a daily dose of insulin regardless of how well controlled his diabetes appeared to be.

DISCUSSION

Goldner and Ricketts (13), in 1942, stated that lapse of treatment seems to be important for the development of insulin allergy. They reviewed 15 cases reported in the literature and indicated that 9 of these cases had had previous periods of insulin treatment with intervals lasting from a few months to several years. They also stated that during the first course of treatment, the development of insulin allergy seems to be characterized

Thus, approximately one in a thousand will have a generalized reaction. Reviews of the case reports in the literature indicate that until recent years, only 63 definite cases with generalized reactions have been reported. At the last (1948) meeting of the American Diabetic Association in Chicago considerable discussion of this problem occurred with both allergists and diabetic specialists participating, and a plea was made that all cases of generalized reactions be reported in the literature so that a large enough series of cases could be accumulated to clarify many of the points in discussion.

At Hines Hospital, 2,949 diabetic patients have been admitted in the last eight years. We have treated just 2 cases of generalized reaction to insulin.

CASE REPORTS

Case 1: BAW, a 53-year-old white nurse first noted glycosuria during a routine urinalysis in 1928. A sister was also a diabetic. She stated that her diabetes had been controlled by diet alone and she had never taken insulin prior to her first admission to this hospital on January 10, 1938. Her blood sugar on admission was 181 mg. per cent. She was placed on a 1700 calorie (194 Gm. CH) diet and discharged eleven days later with a fasting blood sugar of 142 mg. per cent and was not given insulin. She was again admitted to the hospital on January 13, 1942, because of a furunculosis of the left popliteal region. On February 12, 1942, she was started on protamine zinc insulin, 16 units every morning, and on February 18, 1942, local pruritus was noted. On February 26, a 10 cm. reddened area at the site of the injected PZI was noted and the medication was changed to crystalline insulin, 16 units every morning. The difficulty subsided and following the clearing of the infectious process, her insulin requirements dropped and she was discharged on April 7, 1942, without insulin. She was again admitted in October 1942 for treatment of injuries suffered in an automobile accident, and the control of her diabetes appeared adequate without the use of insulin.

She was readmitted to this hospital on January 21, 1944, because of polyuria, polydipsia and severe neuritic pain in the extremities. Her fasting blood sugar was 200 mg. per cent. Because of the previous history of urticaria following insulin injections, she was cautiously started on crystalline zinc insulin, $v.4-4-4$ daily, on March 9. Ten days later a diffuse erythematous rash was noted on the face, neck and ears and on the following day giant urticaria appeared over the same areas and on the body and extremities as well. Insulin was stopped and the urticaria subsided in two days. However, her fasting blood sugars ranged between 275 and 375 mg. per cent and on May 1 she was given a test dose of 2 units of crystalline insulin. Within one hour giant urticaria with marked pruritus again appeared and gradually disappeared within the next few days. An attempt was then made to desensitize her to insulin. Three times daily she was given $1/5$ unit crystalline zinc insulin well diluted with distilled water. This dose was doubled every three days until she could tolerate 13 units three times daily without reaction. She was discharged on June 30, 1944, taking 12-0-12 units daily with excellent control of her diabetes. She has had two subsequent readmissions to this hospital, the last in September 1946. Both were for the treatment of acute infections. She has been taking insulin regularly and has had no further allergic reactions.

ported good results in eliminating local reactions by daily oral administration of 45 to 80 units of histaminase.

Leavitt and Gastineau (20) advocate the mixing of 0.5 to 1.0 cc. of a 1:1000 solution of diphenhydramine hydrochloride (benadryl) with the daily dose of insulin in the treatment of instances of local allergic reactions, reserving oral administration of the antihistaminic drug for the severe generalized reactions. Klein (21) reported the successful use of pyribenzamine hydrochloride in the treatment of a severe generalized reaction. He observed the interesting phenomenon that his patient was completely asymptomatic when given a daily morning injection of PZI and a noon injection of regular insulin covered by daily oral administration of 300 mg. of pyribenzamine. Discontinuance of the pyribenzamine resulted in an immediate recurrence of allergic symptoms. Then over a period of four weeks, the amount of pyribenzamine was gradually reduced until none was being used. With each reduction, there was noted local erythema, induration, and pruritus. Following this, the patient was asymptomatic for a month and then there was an occasional episode of local reaction which was controlled by a single daily dose of 25 to 50 mg. of pyribenzamine.

No immediately fatal reactions have been reported, although several deaths have been attributed indirectly to insulin allergy. Goldner and Ricketts (13), in commenting upon the discouraging results of treatment, state that it is a rather fortunate coincidence that the diabetes in most of these patients is of only moderate severity and with some effort can be controlled by diet alone. Although this is a comforting statement, it must be remembered that in the presence of infections, usually diabetic control is difficult to maintain without the use of insulin and if at such times insulin cannot be given the patient, there may readily be a fatal termination. As an example, our first patient following her desensitization had two subsequent admissions, both for rather severe acute infections. If it had been impossible to give her insulin at these times it is probable that she would not have survived the infection. The relative ease with which she was "desensitized" and the absolute lack of symptomatology subsequently makes us wonder whether or not she represents a case of spontaneous desensitization as described by Harten and Walzer (6) and whether her apparent response to the method of desensitization might have been merely coincidental. The second patient apparently represents an example of true hypersensitivity to insulin protein as evidenced by the skin tests as well as by the fact that upon desensitization to protamine zinc insulin, it was also possible to supplement his PZI with crystalline insulin without initiating an allergic response. It is interesting to note that prior to desensitization no benefit was obtained from the use of antihistaminic drugs; yet,

by a latent period varying from seven to twenty-five days, and they feel that the allergic predisposition apparently needs a period of sensitization for its activation.

Many methods of therapy have been advocated for the treatment of insulin allergy. The patient with mild local reactions often spontaneously desensitizes himself by simply persisting in the use of the kind of insulin to which he is sensitive. Other factors responsible for local reactions, such as chemical irritants and impurities in the manufacture of commercial insulin, have largely been eliminated by improved methods of extraction and purification. Mild local reactions can frequently be eliminated by injecting the insulin deeply into the subcutaneous or intramuscular tissues so that none gets into the skin. Harten and Walzer (6) feel that one of the most effective means of eliminating both local and general reactions is to change the brand of the insulin to one derived from another species or to employ crystalline insulin. There can be no doubt that changes in the preparation employed may overcome a nonspecific etiologic factor in some cases, or even a specific factor, such as sensitivity to the animal which is the source of the insulin. It is not likely to succeed in the presence of a true hypersensitivity to the insulin protein.

Specific desensitization has been used by numerous observers in a variety of methods. Allan and Scherer (11), Bayer (12), and Corcoran (14) have described methods of rapid desensitization in efforts to build up tolerance to insulin in one day. The Bayer method consists in intracutaneous injection, starting with 1/1000 unit and doubling the dose every 15 to 30 minutes as rapidly as the patient will tolerate it without allergic reaction, until a dose of 1 unit is reached. Subcutaneous doses can then be given as tolerated. Herold (15) reported a case in which desensitization was carried out over a period of three months. Another method is to give a very small dose of insulin one-half hour prior to the regular dose. Goldner and Ricketts (13) reported that in 6 out of their own 8 cases and in 11 of 15 cases reviewed in the literature, these procedures were unsuccessful and treatment had to be discontinued because of persistence or recurrence of allergic symptoms. Harten and Walzer (6) also point out that the loss of allergy to insulin may occur spontaneously and hence not all of the desensitizations reported in the literature can be accepted as authentic examples of the direct effects of specific therapy.

Nonspecific measures have also been advocated. Hunscheidt (16) suggested injection of calcium with the insulin. Strasser (17) administered calcium prior to the insulin. Collens, Lerner, and Fialka (10), and Roth and Rynearson (18), reported relief with subcutaneous injection of gradually increasing doses of histamine. Roth, Rynearson, and Horton (19) have re-

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during his second course of treatment, only partial relief was obtained following desensitization, with excellent additional relief following the use of pyribenzamine. This patient also illustrates the important fact emphasized by Dr. Howard Root, that if one discovers a patient who is hypersensitive to insulin and manages to bring this difficulty under control, the patient should be advised to continue taking a small daily dose of insulin in order to maintain his desensitized state, even though his control appears adequate on diet alone. For, should the desensitization be allowed to lapse and the patient subsequently, for the usual variety of causes, develops diabetic acidosis, the one drug which would save his life, insulin, would not be available to him and might conceivably cause his death. We feel that since the advent of the antihistaminic drugs the outlook for these patients has immeasurably improved, since we can now combine one of the several methods of desensitization with the use of one of the antihistaminic drugs in order to control the symptoms during the acute phase and permit desensitization to be established. Once established, continued daily use of insulin would appear to be necessary in order to maintain the state of desensitization.

SUMMARY

1. The incidence of generalized allergy to insulin is small. Reports in the literature indicate that a generalized reaction develops in approximately 1 out of every 1,000 persons using insulin. In a large general hospital we have seen only 2 cases out of 2,949 admissions for the treatment of diabetes mellitus in the last eight years.

2. The importance is emphasized of attempting to desensitize such patients rather than relying on dietary control of the diabetes, since subsequent development of diabetic acidosis in the patient who is allergic to insulin may deprive him of the one therapeutic agent which could save his life.

3. The advent of antihistaminic drugs enables the physician to control more readily the patient's symptoms during the process of desensitization.

4. The importance of continued daily administration of insulin to prevent a lapse in the desensitized state is emphasized.

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CASE REPORT

M. E. (HH 4-188), a 27-year-old, white female was admitted to the hospital June 2, 1947, because of pain in the right lower quadrant of the abdomen and a weight loss of 40 pounds during the past six months. She was known to be about two and one-half months pregnant, her last menstrual period having occurred March 10, 1947. She had no symptoms of thyroid disease until one year before admission, when excessive nervousness and gross tremor of the hands was noted. There was a 40-pound weight loss (165 to 125 pounds) in spite of a good appetite. She always felt warm and used fewer covers than her husband.

Her past history revealed chorea at the age of 14, when she was hospitalized for three months. She was never told of any heart involvement at that time nor during the course of four pregnancies. Her menses were regular, occurring every twenty-eight to thirty days. She had four children; three were full term and one premature. All were living and well. Family history revealed that her mother had a goiter removed.

Physical examination: Pertinent physical findings revealed an excessively nervous, white female with marked psychomotor activity. Her temperature was 98.6 F., pulse 120, respirations 25, and B.P. 150/80. The skin was warm without any unusual perspiration. The eyes had a staring appearance with wide palpebral margins. The thyroid was diffusely enlarged with a marked thrill and bruit over both lobes. Examination of the heart revealed sinus tachycardia; no thrills or murmurs were present. There was mild tenderness in the right lower quadrant of the abdomen. Pelvic examination revealed a corpus enlarged to about a three-months pregnancy, with tenderness over the right adnexa. Marked tremor on extension of the fingers was noted.

Laboratory data: The hemogram showed red cells, 5,500,000; hemoglobin 14 Gm.; and white blood count 5,600, with a normal differential. The urine was normal, the serum cholesterol level was 179 mg. per cent, the B.M.R. was plus 70 per cent and the blood Kahn was negative. X-ray examination showed a normal chest.

Diagnosis: Diffuse thyroid hyperplasia with thyrotoxicosis and pregnancy.

The patient refused to remain in the hospital for further care and was discharged June 7, 1947, to be followed in the outpatient clinic. She was told to take thiouracil, 0.2 Gm. three times daily. Blood counts were done at each weekly visit and at no time did the white count fall below 4,800. The differential count was always within normal limits.

On July 17, 1947, her pulse was 116, and her medication was changed to 50 mg. of propylthiouracil three times daily. On August 15, 1947, her B.M.R. was plus 50 per cent and the electrocardiogram revealed a sinus tachycardia of 110. She was again seen November 28, 1947 (three days before delivery) and her pulse was 96, B.P. 145/80, and weight 156 pounds. The same dosage of propylthiouracil was continued. On December 1, 1947, spontaneous labor occurred and she was delivered of a normal, full-term, baby girl at another hospital. The delivery and puerperium were uneventful. The child was immediately given out for adoption according to a prearranged plan. The foster parents were made known to the writer, and the child has been followed for a period of sixteen months. During this interval the child has developed normally and presents no evidence of a hypothyroid state.

DISCUSSION

The normal birth and subsequent observation of a normally developing child for a sixteen-month period is in accord with the few previous reports of the administration of antithyroid drugs in pregnancy complicated by by hyperthyroidism (4, 5, 6, 7).

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ADDENDUM

Postpartum Course of Patient

The patient was next seen in the clinic on January 30, 1948. The nervousness and tremor had increased, even though she continued to take 50 mg. of propylthiouracil three times daily. She weighed 139 pounds, her pulse was regular with a rate of 120, and

TABLE 1. RÉSUMÉ OF CLINICAL COURSE OF PATIENT FOLLOWING
SECOND DISCHARGE FROM HOSPITAL

Date	Weight (pounds)	Pulse	WBC*	Propyl- thiouracil (mg.)	Remarks
5-21-48	152	84	6400	100 4 times daily	Bruit and thrill over left lobe. Lugol's solution, 5 drops 3 times daily.
6 -4-48	151		9900	100 4 times daily	
6-25-48	144	115		100 4 times daily	Bruit and thrill over both lobes.
7- 9-48	147		6800	100 4 times daily	Nervousness increasing.
8- 6-48	138	115	5800	100 5 times daily	
8-20-48	139	100	4100	100 5 times daily	Still taking Lugol's solution, 5 drops 3 times daily
9-10-48	138	104	4600		Still toxic.
9-17-48	141	128			Bruit and thrill over left lobe.
9-24-48	140	132		200 3 times daily	Serum cholesterol, 189 mg. per cent.
10- 1-48			4200	200 3 times daily	B.M.R. plus 95 per cent.
10-22-48	141	116	4800	200 3 times daily	Protein-bound blood iodine 13.3 micrograms per cent (normal, 5 to 7).
11- 5-48	140	116	5100	100 3 times daily	Bruit and thrill subsiding.
11-12-48	142	112		100 3 times daily	
11-26-48	142	110		100 3 times daily	Nervousness subsiding.
1- 7-49	135	120		100 4 times daily	
1-25-49			5100	100 4 times daily	B.M.R. plus 100 per cent.
2-18-49	138	115		100 4 times daily	Thyroid twice normal size.

* Differential always normal.

The comparatively long follow-up in this case shows that the child has a normally functioning thyroid and that neither thiouracil nor propylthiouracil altered the embryonic development and postnatal function of the gland. This fact can be established only by long term follow-up of such cases.

Chapman *et al.* (8) demonstrated that the fetal thyroid starts to take up radioactive iodine and probably begins to function by the fourteenth week. It may then be argued that in this case the antithyroid drug was not given until about the fourteenth week, when the fetal gland was already developed and beginning to function. Hence, the drug could not alter the embryonic development of the gland, but could merely suppress its in-utero function. Williams (4) has reported several cases in which the mother was treated before and during the entire pregnancy with thiouracil, without any harmful effect on the child. This observation supports the contention that the danger of these drugs to the fetus lies in suppression of the thyroid in-utero rather than in altering the embryonic development of the gland. The danger to the fetus appears to be from overdosage of the mother, resulting in the production of a hypothyroid state with insufficient thyroid hormone (maternal and fetal) to take care of the in-utero requirements (1).

Rune Frisk and Josefsson (9) reported a case of agitation, exophthalmos and low blood cholesterol in a child born of a hyperthyroid mother treated with methylthiouracil. This was attributed to an overactive anterior pituitary, caused by suppression of the fetal thyroid hormone by the drug. Although this was not proven, it suggests another possible danger in the use of these drugs during pregnancy.

SUMMARY

A case of thyrotoxicosis complicating pregnancy treated with thiouracil and propylthiouracil is reported. The child was followed for sixteen months and developed normally showing no ill effects from the drug. The potential hazards of the goitrogens to the fetus is discussed.

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groups and, since osteitis deformans is a disease of middle and old age, studies using these tests must be compared to control series of normal subjects from similar age groups.

In other unpublished experiments, however, the influence of elevated serum alkaline phosphatase on carbohydrate metabolism in dogs was studied. The intravenous injection of a potent phosphatase solution prepared from intestinal mucosa produced serum alkaline phosphatase levels varying from 37 to 180 Bodansky units per 100 cc. with a gradual fall to normal levels in from one and one-half to three hours. Both oral and intravenous glucose tolerance tests performed during such a period of experimental hyperphosphatasemia were grossly elevated and prolonged as compared to control tests prior to the injection of phosphatase. The results of a typical experiment are as follows:

*Control glucose tolerance test—0.5 mg. glucose per kilo
intravenously in an 8-kilogram male dog.*

Minutes	0	15	30	60	75	90
Blood sugar (mg. per 100 cc.)	92	145	106	88	83	90
Serum alkaline phosphatase (Bodansky units per 100 cc.)	9.6	—	—	—	—	—

*Repeat glucose tolerance test after the intravenous injection of
3 cc. of phosphatase solution (200 Bodansky units per cc.)*

Minutes	0	15	30	60	75	90
Blood sugar (mg. per 100 cc.)	93	328	234	168	137	—
Serum alkaline phosphatase (Bodansky units per 100 cc.)	8.0	91.4	73.9	24.1	17.5	—

The discrepancy between the clinical and experimental results remains unexplained. In several of our patients serum alkaline phosphatase levels were as high as those produced experimentally in dogs.

This brief presentation of our findings in Paget's disease does not, of course, alter the significance of the symptomatic response described by Moehlig and it is our purpose to investigate its effect in our own patients.

NORMAN G. SCHNEEBERG, M.D.
June 22, 1949.

1930 Chestnut Street,
Philadelphia 3, Pa.

her B.P. was 145/80. The serum cholesterol was 200 mg. per cent, and the B.M.R. plus 44 per cent. Propylthiouracil was increased to 50 mg. four times daily. On March 19, 1948, she weighed 130 pounds, her pulse was 120, and her B.P. 145/80. The same dosage of the drug was continued.

(Second admission) The patient re-entered the hospital April 20, 1948 because of pain in the chest, back, and shoulders. On the day before, she had been seen in the admitting room because of the above complaints. Examination revealed auricular fibrillation with a rate of 120 and a pulse deficit. Hospitalization was advised, but she refused. Digitoxin was prescribed by the admitting room physician. She returned the next day because the chest pain became more severe after taking a total of 0.6 mg. of digitoxin.

Physical examination revealed her to be very apprehensive, and perspiring with a normal temperature and respiratory rate of 28. The staring of the eyes and widened palpebral margins were unchanged from the previous admission. The thyroid was diffusely enlarged with a loud bruit and thrill over both lobes. Examination of the heart revealed the apical impulse to be in the fifth interspace on the anterior axillary line. There was auricular fibrillation with a rate of 140 and an irregular pulse of 110. No thrills or murmurs were present; her B.P. was 140/80.

The hemogram was normal and the serum cholesterol level was 200 mg. per cent. An electrocardiogram revealed a flutter fibrillation with a rate of 140 and a left ventricular strain patten.

The patient was quieted with a sedative and given quinidine and 100 mg. of propylthiouracil three times daily. Conversion to a sinus rhythm occurred the following night. On the morning following conversion, an electrocardiogram showed a sinus rhythm of 100 and left ventricular strain. On April 23, 1948, the propylthiouracil was increased to 100 mg. four times daily. Her pulse varied between 80 and 100. Surgery was advised but she refused. She was discharged on May 11, 1948, taking 100 mg. of propylthiouracil four times daily and Lugol's solution, 5 drops three times daily. She was followed in the out-patient clinic. A résumé of her clinical course from the time of discharge to the present, is shown in Table 1. She has continued to take 400 mg. of propylthiouracil daily until the present time but the disease is still not under complete control.

Letter to the Editor

GLUCOSE TOLERANCE IN OSTEITIS DEFORMANS

TO THE EDITOR:

THE April 1949 issue of the Journal contains a letter by Dr. Robert C. Moehlig of Detroit advocating the administration of insulin for the relief of pain in patients with osteitis deformans. The rationale of this therapy was based on the finding of lowered sugar tolerance in the majority of patients with Paget's disease of bone.

We have investigated glucose tolerance in 11 patients with osteitis deformans by both the oral and intravenous tests and have failed to find any significant statistical difference between their glucose tolerance curves and those obtained from a control series of patients over 50 years of age. Both oral and intravenous glucose tolerance is depressed in older age

The 1950 Awards of the Association for the Study of Internal Secretions

FELLOWSHIPS

THE AYERST, MCKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Lawrence Wilson.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed \$2,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AWARDS

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of \$1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945

The Association for the Study of Internal Secretions Request for Biographical Data for New Roster

The membership roll of the Association is being revised in preparation for the issuance of a new Roster as of December 31, 1949. *Copy for this must be in the hands of the printer not later than October 1, 1949.*

Return postal cards are being mailed to members requesting the following information:

Name.....	Degrees.....
Mailing Address.....	
.....	
Teaching or Research Position:.....	
.....	
.....	
If in active practice of medicine, specialty:.....	
.....	

Members are urged to complete and return the card immediately and to keep the Secretary informed at all times of any change in the above.

HENRY H. TURNER, M.D.
Secretary-Treasurer
1200 North Walker Street
Oklahoma City 3, Oklahoma

The 1950 Meeting of the Association for the Study of Internal Secretions

The next Annual Meeting of the Association will be held in San Francisco, June 23 and 24, 1950. The hotel in which we shall meet has not yet been determined because of the uncertainty of AMA commitments, but it will be announced in an early issue of the Journal. Members are urged to make their hotel reservations as soon as possible.

The 1950 Meeting of the American Goiter Association

The next meeting of the American Goiter Association will be held at the Hotel Shamrock, Houston, Texas, March 9, 10, and 11, 1950. It is recommended that all physicians wishing to attend make their hotel reservations early.

Award of the American Goiter Association

VAN METER PRIZE

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Houston, Texas, March 9, 10 and 11, 1950, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed three thousand words in length, and must be presented in English. A typewritten double spaced copy *in duplicate* should be sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East St. Vrain Street, Colorado Springs, Colorado, not later than *January 15, 1950*. The Committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or pre-clinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for \$1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to \$1,800.

* * * * *

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950*.

of every guinea pig were made from three days prior to thyroidectomy through the period of injection and again fifteen minutes post mortem. For the purpose of measuring this distance a new instrument was devised which has certain advantages over those previously described in that it is very simple to operate, requires no special placing or bright lighting of the subject animal and is accurate to 0.1 mm. It consists essentially of two pairs of calipers in the form of steel rods, 2 mm. in diameter and pointed

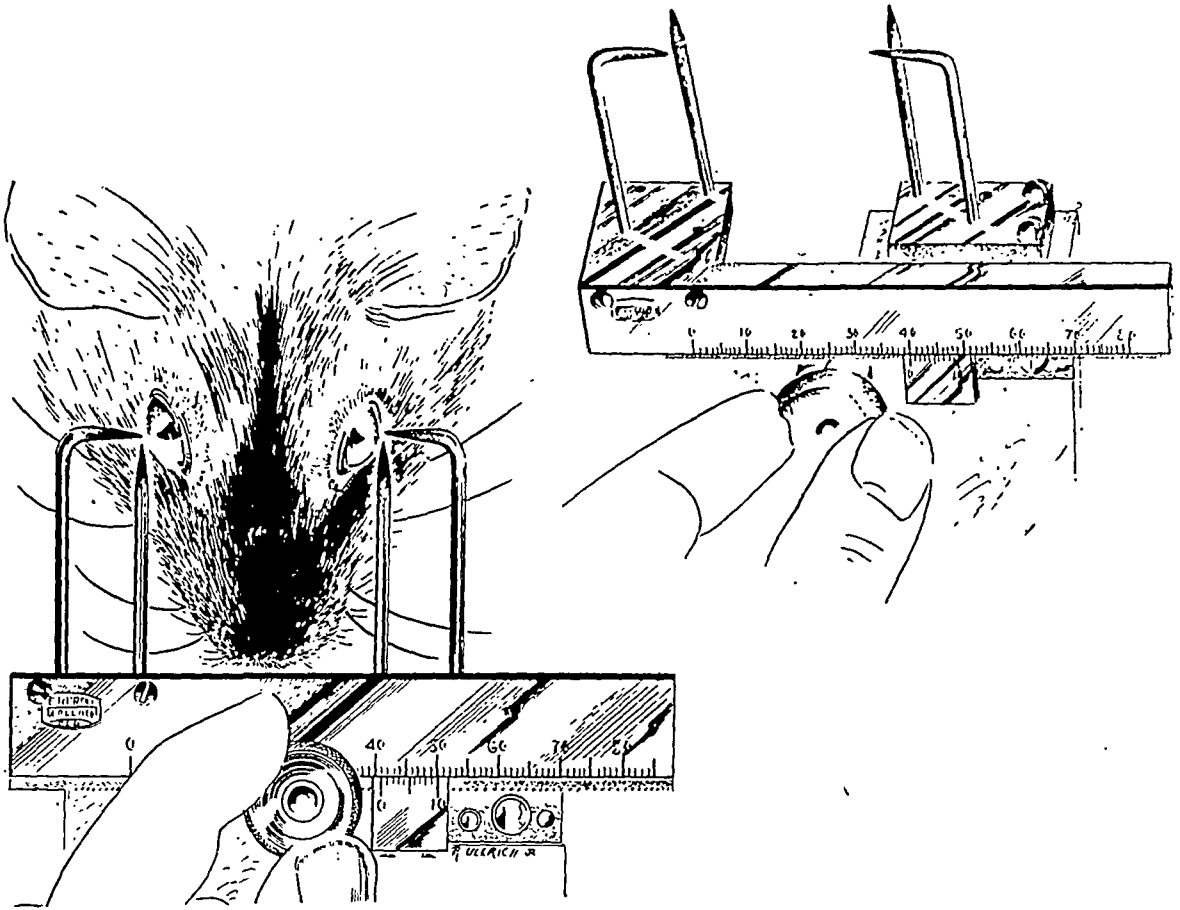


FIG. 1. Instrument for measuring intercorneal distance (ICD) in animals.

at the ends, with a rack and pinion adjustment and a vernier scale (Fig. 1). The calipers are arranged so that one pair is situated above the other, the upper set being more widely spaced than the lower, but bent at right angles near the tips so that their points directly overlie the points of the lower set opposite the respective zero points on the vernier scale. By having two fixed points in a vertical line on the instrument to line up with each of the animal's lateral corneal surfaces an accurate vertical alignment is assured on every measurement without the necessity of the pointers touching or being held very close to the eyes. The lower pointers are usually held 1 or 2 cm. above the lateral corneal surfaces with which they are being aligned. Such an instrument not only can measure accurately to 0.1

TABLE 1. THE EFFECT OF VARIOUS DOSAGES OF PITUITARY EXTRACT UPON THE PRODUCTION OF EXOPHTHALMOS AND THE MOBILIZATION OF FAT IN INTACT AND THYROIDECTOMIZED GUINEA PIGS.

GP No.		Injection*	Body weight in grams**	Liver fat (histol'y)	Ante-mortem ICD changes (mm.)†				Post-mortem ICD changes (mm.)‡		
					Meas.	Time† (hours)	Cor-rection for body weight	Cor-rected	Meas.	Cor-rection for body weight	Cor-rected
1	Thyroid-ectomized	2 cc. normal saline	265	0	+0.7	48	-0.6	+0.1	-0.3	-0.6	-0.9
2	Thyroid-ectomized	2 cc. normal saline	232	0	+0.6	24	-0.3	+0.3	-0.3	-0.5	-0.8
3	Thyroid-ectomized	Pit. ext. = 50 u TSH	280	2 +	+2.0	48	+0.2	+2.2	+1.0	+0.2	+1.2
4	Thyroid-ectomized	Pit. ext. = 25 u TSH	285	2 +	+2.8	24	-0.2	+2.6	+2.0	0	+2.0
5	Thyroid-ectomized	Pit. ext. = 25 u TSH	265	2 +	+1.3	48	+0.1	+1.4	+1.1	+0.1	+1.2
6	Thyroid-ectomized	Pit. ext. = 10 u TSH	305	2 +	+2.6	24	+0.2	+2.8	+0.6	+0.1	+0.7
7	Thyroid-ectomized	Pit. ext. = 10 u TSH	285	3 +	+2.5	24	0	+2.5	+1.4	+0.2	+1.6
8	Thyroid-ectomized	Pit. ext. = 5 u TSH	283	3 +	+2.0	24	-0.6	+1.4	+0.6	0	+0.6
9	Thyroid-ectomized	Pit. ext. = 5 u TSH	296	4 +	+1.7	24	-0.2	+1.5	+0.1	+0.1	+0.2
10	Thyroid-ectomized	Pit. ext. = 1 u TSH	268	1 +	+0.5	48	-0.2	+0.3	-0.4	-0.2	-0.6
11	Thyroid-ectomized	Pit. ext. = 1 u TSH	273	1 +	+0.6	24	-0.6	0	-0.2	-0.6	-0.8
12	Intact	2 cc. normal saline	258	0	-0.2	24	-0.3	-0.5	-1.5	-0.6	-2.1
13	Intact	2 cc. normal saline	240	0	+0.3	48	-0.7	-0.4	-1.1	-0.7	-1.8
14	Intact	Pit. ext. = 50 u TSH	238	2 +	+1.8	24	0	+1.8	+0.7	+0.5	+1.2
15	Intact	Pit. ext. = 50 u TSH	287	3 +	+2.7	24	0	+2.7	+1.0	+0.5	+1.5
16	Intact	Pit. ext. = 25 u TSH	253	4 +	+2.9	24	-0.1	+2.8	+1.7	+0.4	+2.1
17	Intact	Pit. ext. = 25 u TSH	222	3 +	+0.6	48	+0.6	+1.2	-0.8	+0.6	-0.2
18	Intact	Pit. ext. = 10 u TSH	239	3 +	+1.8	24	-0.2	+1.6	-0.1	+0.2	+0.1
19	Intact	Pit. ext. = 10 u TSH	195	2 +	+2.9	48	+0.2	+3.1	+0.2	+0.2	+0.4
20	Intact	Pit. ext. = 5 u TSH	240	3 +	+1.5	24	0	+1.5	+0.2	+0.3	+0.5
21	Intact	Pit. ext. = 5 u TSH	265	4 +	+0.9	48	+0.2	+1.1	-1.4	+0.2	-1.2
22	Intact	Pit. ext. = 1 u TSH	247	0	+0.5	48	-0.3	+0.2	-1.0	-0.3	-1.3
23	Intact	Pit. ext. = 1 u TSH	227	0	+0.7	48	-0.4	+0.3	-1.0	-0.4	-1.4

* Animals received 2 daily subcutaneous injections of the doses indicated, and were sacrificed on the third day.

** At the time of the first injection.

† Time after first injection at which intercorneal distance (ICD) measurement was greatest (hours).

‡ As compared with pre-injection measurements.

in animal #19, one which showed only 2+ fat in the liver. Nevertheless, every animal which showed exophthalmic effect in this experiment also showed some degree of increased fat infiltration and neither effect was present at the 1-unit dosage.

A comparison of the exophthalmic effects persisting post mortem reveals essentially the same correlation and confirms the findings observed during life. Both saline-injected and pituitary-injected guinea pigs experienced a decrease in intercorneal distance fifteen minutes post mortem as compared with measurements made just prior to death, but a comparison of post-

TABLE 2. THE EFFECT OF DOSES OF PITUITARY EXTRACT CONTAINING BETWEEN 1 AND 5 UNITS OF THYROTROPIC ACTIVITY UPON THE PRODUCTION OF EXOPHTHALMOS AND FAT DEPOSITION IN THE LIVER, IN INTACT GUINEA PIGS.

GP No.	Injection*	Body weight in grams**	Liver fat (histol'y)	Ante-mortem ICD changes (mm.)‡				Post-mortem ICD changes (mm.)‡		
				Meas.	Time† (hours)	Correc- tion for body weight	Cor- rected	Meas.	Cor- rected for body weight	Cor- rected
24	Pit. ext. =5 u TSH	208	2+	+1.6	24	-0.2	+1.4	+0.2	+0.1	+0.3
25	Pit. ext. =5 u TSH	194	2+	+2.3	24	-0.1	+2.2	0	+0.3	+0.3
26	Pit. ext. =4 u TSH	223	3+	+2.1	24	-0.2	+1.9	+0.3	+0.2	+0.5
27	Pit. ext. =4 u TSH	176	1+	+0.9	24	-0.2	+0.7	-0.5	0	-0.5
28	Pit. ext. =3 u TSH	175	1+	+1.8	24	-0.3	+1.5	-0.5	+0.1	-0.4
29	Pit. ext. =3 u TSH	222	2+	+1.1	24	0	+1.1	0	+0.2	+0.2
30	Pit. ext. =2 u TSH	234	2+	+0.6	24	-0.2	+0.4	-0.8	0	-0.8
31	Pit. ext. =2 u TSH	215	2+	+0.2	24	+0.1	+0.3	+0.2	+0.1	+0.3
32	Pit. ext. =1 u TSH	201	0	+0.8	48	0	+0.8	0	0	0
33	Pit. ext. =1 u TSH	243	0	+0.1	48	+0.1	+0.2	-0.6	+0.1	-0.5

* Animals received 2 daily subcutaneous injections of the doses indicated, and were sacrificed on the third day.

** At the time of the first injection.

† Time after first injection at which intercorneal distance (ICD) measurement was greatest (hours).

‡ As compared with pre-injection measurements.

mortem readings with pre-injection measurements indicated whether exophthalmos persisted after death.

It will be noted that guinea pigs #17 and #21 did not show persistence of exophthalmos post mortem to a significant degree and these two animals also exhibited a comparatively small exophthalmic effect during life, but their livers showed 3+ and 4+ fat respectively. Hence they strengthen the impression that the degree of fat deposition in the liver following pituitary injections is not necessarily correlated with the degree of exophthalmos produced.

The second portion of this study was undertaken in order to determine

at what dose between the levels of 5 and 1 units of TSH the exophthalmic and fat-mobilizing effects disappeared. The results are presented in Table 2.

It may be seen that histologically demonstrable fatty infiltration of the liver was present with a dosage as low as 2 units, but none occurred at the 1-unit level. Definite exophthalmic effect, however, was not evident below the 3-unit level. The proptosis produced with these smaller doses of pituitary extract was more transient than that produced with larger amounts, reaching a maximum twenty-four hours after the first injection and showing considerable decline at the forty-eight hour measurement. This probably accounts for the finding that post-mortem readings failed to show a consistent correlation with the ante-mortem exophthalmic effect, since exophthalmos was subsiding rather rapidly by the time the animals were sacrificed.

It will be noted that the response of animals #27 and #28 was different from that of the other animals in their respective groups. They both showed slight exophthalmos at the 24-hour measurement, but this subsided rather rapidly so that there was no persistence post mortem, and there was no significant increase in fat in their livers. This behavior may have been related to the fact that both of these guinea pigs were considerably underweight as compared with the other animals in this experiment at the time injections were started.

DISCUSSION

Although the number of animals studied at each dosage level was small, the results were sufficiently clear cut to be informative. Not only did thyroidectomy fail to enhance the production of exophthalmos or the histologically demonstrable mobilization of fat to the liver which occurs within forty-eight hours following pituitary injections in young guinea pigs, but there was no apparent correlation between the degree of exophthalmos and the degree of fatty infiltration of the livers. Nevertheless, the smallest doses of extract which produced detectable effects with regard to these two phenomena were quite close. Hence, although this study indicates that the degree of exophthalmos is not dependent upon the degree of fat mobilization, it does not prove that these two effects are unrelated.

The failure of thyroidectomy to affect the exophthalmos may have been related to the fact that these guinea pigs did not lose weight during the 48-hour period following pituitary injections. In this respect it is interesting to note that the only animal which showed considerable weight loss during the period of injection was the one which showed the smallest degree of exophthalmos when injected with a daily dose containing 10 or more units of TSH. The results of this study are therefore not incompatible with those of Pochin (12), who reported that the enhancing effect of thyroidectomy

side earlier. In Figure 2 the changes in intercorneal distance have been charted graphically for the sake of comparison.

It appears, therefore, that iodination at pH 4.2 produced over 95 per cent inactivation of the thyrotropic principle, but demonstrable exophthalmic and fat-mobilizing effects persisted. Furthermore, gonadotropic ac-

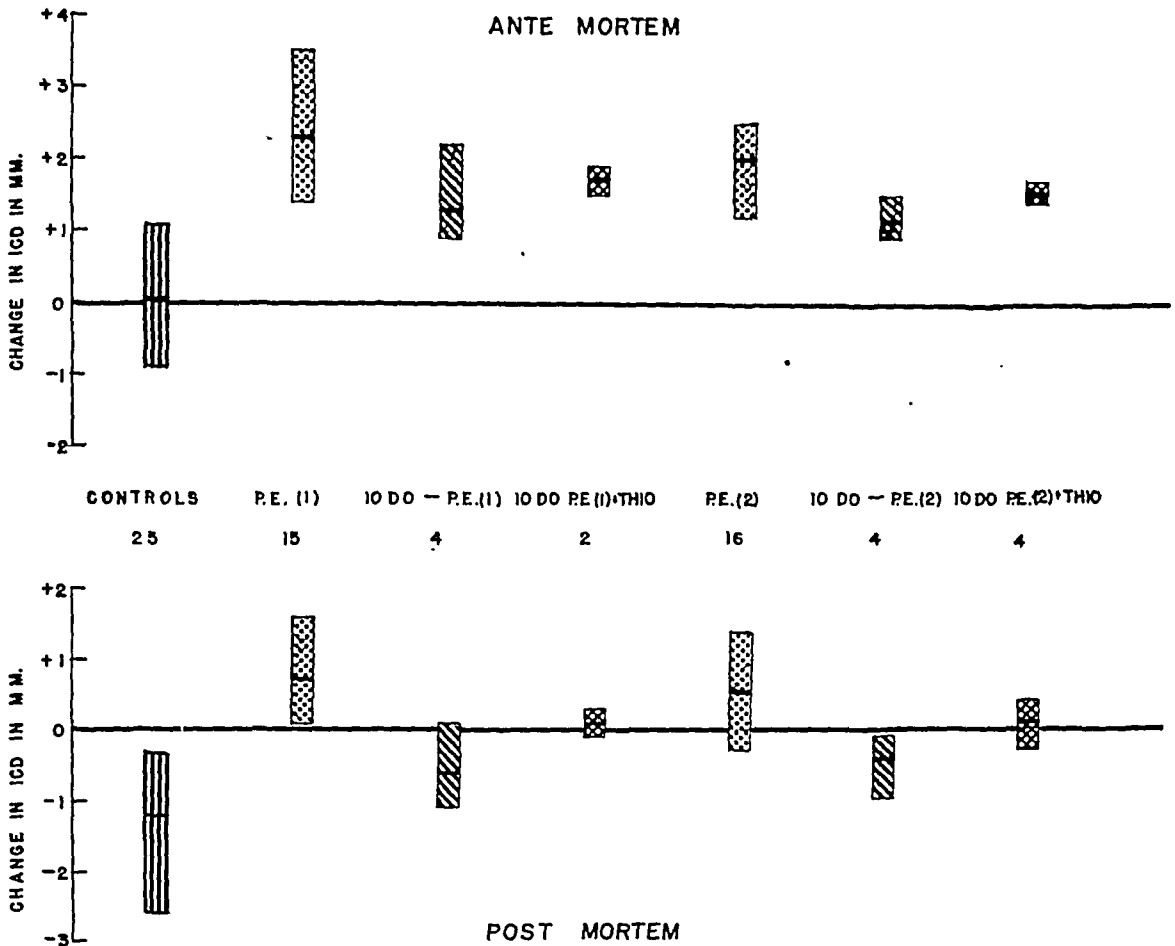


FIG. 2. A comparison of the exophthalmic effects of untreated pituitary extract, of iodinated pituitary extract (at pH 4.2) and of the iodinated extract after treatment with thiouracil in intact young guinea pigs. Each preparation was administered in two daily doses which originally contained 10 units of thyrotropic activity in the untreated state. The number of animals studied in each group is indicated under the type of treatment. The horizontal line through each column represents the mean, and the column itself, the range between the maximum and minimum effects.

tivity, as determined by chick testis weights, was partially decreased by such iodination. Injection of dilute Lugol's solution and pituitary extract at separate subcutaneous sites into both chicks and guinea pigs resulted in no impairment of thyrotropic, exophthalmic, or fat-mobilizing effects during the time required by the respective assays.

Treatment of the iodinated extract with thiouracil resulted in partial restoration of the thyrotropic activity, and probably also of exophthalmic

mals injected with extract #1 and 16 injected with extract #2 in quantities containing 10 units of thyrotropic activity, daily for two days. Statistical analyses of the increases in proptosis, using the "student" formula, indicate a high degree of significance for the exophthalmos produced ante mortem by the iodinated as well as the untreated and the thiouracil-treated extract in comparison with the controls.³ The post-mortem measurements revealed a considerable decrease of intercorneal distance from the maximum ante-mortem reading in all animals as had been noted in the previous study (7) but when the post-mortem measurements were compared with the pre-

TABLE 2. THE EFFECTS OF IODINATION UPON THE EXOPHTHALMIC AND FAT-MOBILIZING PROPERTIES OF PITUITARY EXTRACT IN INTACT GUINEA PIGS.

Group*	No. of animals	Liver fat (histol'y)	Maximum inc. in ICD (mm.)†	Minimum inc. in ICD (mm.)†	Mean	Standard deviation	Value of p‡
<i>Ante mortem:</i>							
2 cc normal saline	25		+1.1	-0.9	+0.06	0.52	
Pit-ext. #1=10 u TSH	15		+3.5	+1.4	+2.3	0.57	<0.001
Iodo-Pit-ext. #1**	4		+2.2	+0.9	+1.28	0.61	<0.001
Iodo-Pit-ext. #1 +Thiouracil**	2		+1.9	+1.5	+1.7	0.28	<0.001
Pit-ext. #2=10 u TSH	16		+2.5	+1.2	+2.0	0.46	<0.001
Iodo-Pit-ext. #2**	4		+1.5	+0.9	+1.15	0.48	<0.001
Iodo-Pit-ext. #2 +Thiouracil**	4		+1.7	+1.4	+1.55	0.11	<0.001
<i>Post mortem:</i>							
2 cc normal saline	19	0 -1 +	-0.3	-2.6	-1.2	0.67	
Pit-ext. #1=10 u TSH	13	3 +4 +	+1.6	+0.1	+0.75	0.44	<0.001
Iodo-Pit-ext. #1	4	0 -2 +§	+0.1	-1.1	-0.62	0.52	<0.2 >0.1
Iodo-Pit-ext. #1 +Thiouracil	2	2 +3 +	+0.3	-0.1	+0.1	0.28	<0.02 >0.01
Pit-ext. #2=10 u TSH	14	3 +4 +	+1.4	-0.3	+0.54	0.54	<0.001
Iodo-Pit-ext. #2	4	3 +	-0.1	-1.0	-0.43	0.4	<0.05 >0.02
Iodo-Pit-ext. #2 +Thiouracil	4	4 +	+0.4	-0.3	+0.08	0.3	<0.001

* Indicated doses were administered subcutaneously for two days and the animals sacrificed on the third day.

** These preparations were administered in daily amounts which had originally contained 10 units of TSH.

† As compared with pre-injection measurements and corrected for changes in body weight.

‡ Values less than 0.01 are of statistical significance.

§ One animal's liver showed no fat, the other three, 2 +.

injection readings the differences between the pituitary-treated and the control guinea pigs became evident. These post-mortem differences were great enough to be of statistical significance in the groups injected with untreated extract, but not in those which received the iodinated preparations, possibly due to the fact that milder degrees of exophthalmos tend to sub-

³ Since the preparation of this table, the exophthalmic effect of iodinated extract has been studied in 16 more guinea pigs, making a total of 24 animals in this group. Statistical analysis of the entire group reveals an even greater degree of significance of the exophthalmos produced, the value of *t* for the entire group being 7.316 as compared with 4.26 and 4.07, respectively, for the two groups of 4 animals which received iodinated extract listed in the table above.

Thus, if thyrotropic activity were responsible for the exophthalmic and fat-mobilizing effects, and if it were present in sufficient quantities in the iodinated extract on the first day or two following iodination, but not thereafter, Groups 1 and 2 of the guinea pigs should show exophthalmos and fatty livers, but Groups 3 and 4 should not. In addition, the average thyroid weight of the chicks in Group D would not be expected to be any greater than that of those in Group C.

The guinea pigs of Groups 3 and 4 did show definite exophthalmos and fatty livers comparable to the effects shown by Groups 1 and 2, however. Hence the iodinated extract must have lost very little of its exophthalmic and fat-mobilizing activity while being kept in the refrigerator for four days.

Furthermore, the average thyroid weight of chick Group D was definitely higher than that of Group C (Table 3). Hence it is unlikely that the iodinated extract retained considerable thyrotropic activity for the first day after preparation.

TABLE 3. THE EFFECT OF IODINATION OF PITUITARY EXTRACT UPON ITS
THYROID-STIMULATING ACTIVITY IN CHICKS.

Group*	Thyroid weight (mg.)
A. Controls (1 cc. distilled water daily)	4.92
B. Pituitary extract #2 (2/3 J-S unit TSH daily)	10.52
C. Iodo-P.E. #2 (10/3 J-S units TSH daily)	6.27
D. P.E. #2 (3 J-S units TSH) + I-KI separately first day, followed by iodo-P.E. (10/3 J-S units TSH) for two days	8.25

* Each group consisted of 10 chicks injected subcutaneously with the indicated doses for three consecutive days and sacrificed on the fourth day.

This experiment also confirms the previous studies, in that a quantity of iodinated extract equivalent to 10 units of original thyrotropic activity (Group C) did not produce as great an increase in thyroid weight as a quantity of untreated extract containing 2 units. The assay consequently indicates that the dose of iodinated extract administered to the guinea pigs contained less than 2 units of thyrotropic activity, a quantity which had been found to be insufficient to produce exophthalmos and fatty infiltration of the liver on the basis of the activity of this principle alone.

DISCUSSION

Although the evidence obtained in these studies is to a great extent indirect, and although the nature of the experiments was such that numerous controls were necessary to evaluate possible extraneous factors, neverthe-

less the results were sufficiently consistent to be reasonably convincing.

Although iodination of pituitary extract at a pH above 5 produced irreversible inactivation of all three principles, iodination at pH 4.2 apparently resulted in less loss of exophthalmic and fat-mobilizing than of thyrotropic activity. Thus, both exophthalmos and fat mobilization were repeatedly produced in guinea pigs by a dose of iodinated extract which contained much less thyrotropic activity by chick assay than was necessary to produce these effects when the untreated extract was administered. Since these effects could not be accounted for on the basis of thyrotropic activity alone, it therefore appears that the role of thyrotropic hormone in experimental exophthalmos is at best an auxiliary one, and the primary factor responsible for the development of this phenomenon remains unidentified. It may be an entirely separate principle of pituitary extract, or it may result from the combined actions of two or more of the recognized principles.

SUMMARY AND CONCLUSIONS

The effect of iodination upon the thyrotropic, exophthalmic and fat-mobilizing principles of pituitary extract has been studied. Thyrotropic activity was assayed in chicks. Exophthalmos and fat mobilization were assayed in young guinea pigs following two daily injections of preparations of pituitary extract. Interocular distances were measured with a simple instrument which is accurate to 0.1 mm., and fat mobilization was determined histologically by Scarlach R stains of frozen sections of the livers.

Iodination at pH 5-6 resulted in irreversible inactivation of the thyrotropic, exophthalmic and fat-mobilizing principles. Iodination at pH 4.2 produced up to 95 per cent loss of thyrotropic activity but sufficient exophthalmic and fat-mobilizing activity remained to produce demonstrable effects in young guinea pigs. Suitable controls indicated that these effects therefore could not be attributed to the thyrotropic principle alone.

Consequently, the role of thyrotropic hormone in experimental exophthalmos following pituitary injections appears to be at best an auxiliary one, and the primary factor responsible for this phenomenon remains unidentified.

Treatment of the iodinated extract with thiouracil partly restored the thyrotropic activity and apparently slightly increased the exophthalmic and fat-mobilizing effects.

Acknowledgment

The author is indebted to Miss Beatrice Lennón, Miss Priscilla Merrill and Miss Olive Joneson for their technical assistance at various times in the course of these studies.

STUDIES OF THE RELATIONSHIP OF THE THYROTROPIC, EXOPHTHALMIC AND FAT-MOBILIZING PRINCIPLES OF PITUITARY EXTRACT

III. THE EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) AND DESOXYCORTICOSTERONE ACETATE (DOCA) UPON EXOPHTHALMOS AND FAT MOBILIZATION IN GUINEA PIGS*§

Van Meter Prize Award Essay

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IN the preceding report (1) it has been found that the exophthalmos produced within forty-eight hours by the injection of pituitary extract into guinea pigs cannot be attributed to thyrotropic activity alone. It has also been found that this exophthalmos can hardly be attributed to pituitary-induced mobilization of fat per se (2), since proptosis developed in some animals which showed no increased fat deposition in their livers. Nevertheless, the exophthalmic and fat-mobilizing activity seemed closely related, disappearing approximately together with diminishing doses of pituitary extract and behaving similarly when the extract was iodinated. It therefore seemed advisable to investigate this relationship further, if possible.

Fry (3) has reported that the fatty livers induced by administration of anterior pituitary extract to intact rats cannot be induced in adrenalectomized animals. More recently, Baker *et al.* (4) have noted that the administration of adrenocorticotrophic hormone (ACTH) to rats on a high carbohydrate diet has been followed by increased histologic fat in the livers. Consequently, it seemed reasonable to suspect that ACTH might be

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§ The investigation was performed during the tenure of a fellowship of the American Cancer Society recommended by the Committee on Growth of the National Research Council.

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thyroid is known, the calculation of total iodine accumulation is a simple matter. Since the kidneys excrete the isotopes indiscriminately, there will be the same proportion of each in the serum and in the urine. Or, expressed in another fashion, $\text{Serum } I^{127}/\text{Serum } I^{131} = \text{Urine } I^{127}/\text{Urine } I^{131}$. At any time then, the specific activity (I^{131}/I^{127}) of the iodide in the urine being formed will be the same as that of the serum. Since arterial blood from the same source supplies both the thyroid and the kidneys and contains identical amounts of iodide, the specific activity of the iodide of the urine excreted over a short period will accurately reflect the specific activity of the iodide entering the thyroid during this same period.

A group of 31 euthyroid and 13 untreated thyrotoxic subjects were studied by this method. In all instances a tracer dose of 100 microcuries of I^{131} was administered by mouth. Within a few minutes the subject urinated and discarded the specimen. During the course of the test enough water was drunk to provide an adequate urinary output. At approximately hourly intervals for six to twelve or more hours the radioactivity in the thyroid gland was measured with a sensitive gamma (Geiger-Müller) counter at a distance of 35 centimeters from the gland. The background (radioactivity over the lower thigh just above the knee) was subtracted from the thyroid count. The absolute quantity of I^{131} in microcuries was calculated by comparing the net count with that resulting from a standard radioactive iodine solution under similar geometric conditions. Nearly simultaneously with each thyroid count, urine and blood specimens were collected.

Astwood and Bissell's modification (2) of Kendall's method was used for the analyses of the urines for stable iodine, I^{127} . The I^{131} content of the urine and serum was determined by comparing aliquots evaporated on flamed copper planchets with appropriately diluted portions of the administered I^{131} , using a thin window beta counter. In order to compensate for the greater self-absorption of the serum specimens, appropriate quantities of normal serum (containing no radioactivity) were evaporated on the discs with the urines and standard solutions.

When the successive values representing accumulated I^{131} in the thyroid were plotted against a "square root of time" scale, the parabolic curves were converted to straight lines in normal subjects (3). The effect of inhibiting agents administered during the tests was evident as deviations from this linear course of accumulation. Thus, in normal subjects, anti-thyroid drugs of various types have been quantitatively assayed by this method (3). In this study, also in euthyroid subjects, the method was utilized in ascertaining the action of the iodide ion. The pattern of iodine collection exhibited by very hyperplastic thyroids such as in thyrotoxicosis could not be fitted to a straight line.

THE DIRECT ESTIMATION OF THE RATE OF THYROID HORMONE FORMATION IN MAN. THE EFFECT OF THE IODIDE ION ON THYROID IODINE UTILIZATION*

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THE interest of thyroidologists was recently aroused by the demonstration by Wolff and Chaikoff (1) that, with levels of serum iodide higher than 20 to 30 micrograms per cent, organic binding of iodine in the rat thyroid was inhibited. Extension of these observations to man was undertaken in view of the paradox thus presented, *i.e.*, that adequate iodide seemed to prevent hormone formation in the rat and yet failed to control thyrotoxicosis completely or to produce myxedema in man.

Although various indirect methods have been used for estimating the rate of hormone formation in man, such as the amount of thyroid necessary to alleviate myxedema, or the urinary excretion of iodine, no direct measurements of this function have been described.

It is the purpose of this paper to present a method for the direct estimation of the rate of iodine utilization by the thyroid in man, and to determine the effects of various amounts of iodide on this process. A simple method for calculating the serum iodide is also described.

METHODS

The principles utilized were, (a) serial quantitative determinations of radioactive iodine uptake by the thyroid, and (b) calculation of the specific activity of the accumulated thyroid iodine by analysis of the simultaneously excreted urine for both I^{131} and I^{127} .

These procedures are based on the assumption that the two isotopes, I^{131} (radioiodine) and I^{127} (stable iodine), chemically identical in the test tube, are metabolized in exactly the same manner in the body. Then, if the quantity of stable iodine accompanying each microcurie of I^{131} into the

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Small amounts of iodide were given (single doses orally of 10 micrograms of potassium iodide— 7.63 micrograms I^- —/kilogram body weight) to 15 additional euthyroid subjects; in 14, this was added to the I^{131} as carrier, and in the other patient was administered during the course of the uptake. In the instances in which they were determined, the serum iodide values were increased 1 to 3 micrograms per cent by this addition. In all except one person, the amounts of total iodide collected by the thyroid were higher than in those not given iodide, or were increased as a result of the

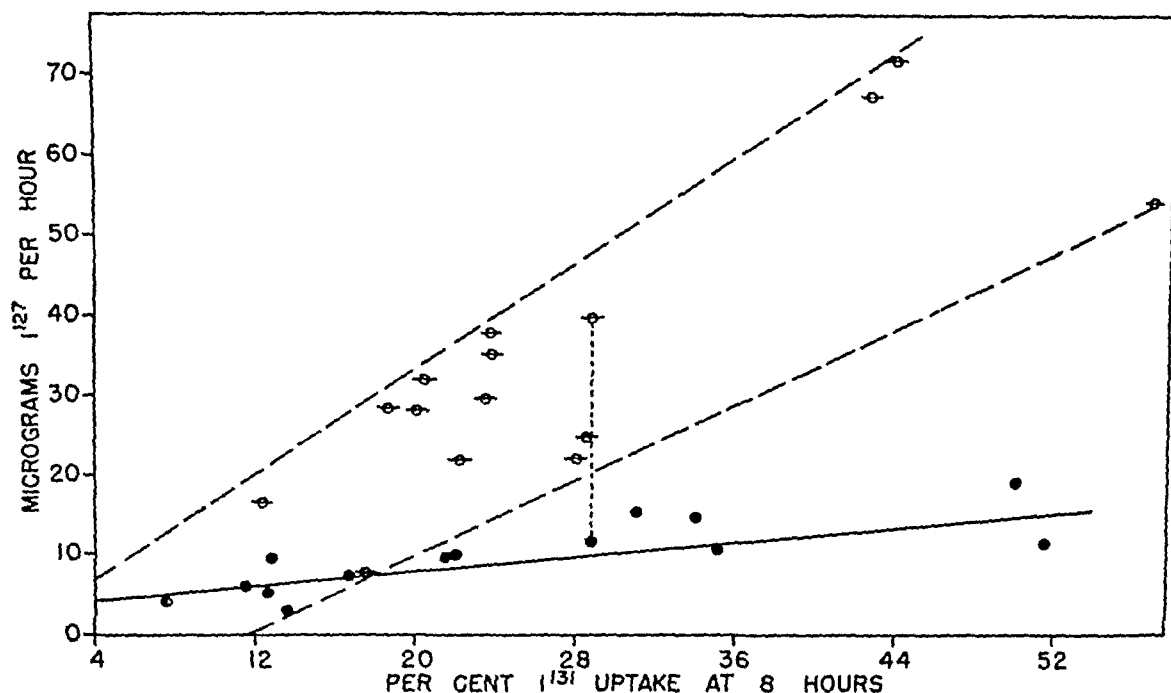


FIG. 1. The rate of total iodine utilization by the thyroid gland plotted against the radioiodine uptake in euthyroid subjects. The per cent of I^{131} collected in the thyroid at eight hours after administration of the dose is selected as an index of radioiodine uptake (abscissa). On the ordinate is represented the rate of I^{127} collection per hour. The solid circles signify that the subjects received no additional iodide; the serum iodide levels were usually less than 1 microgram per cent. The crossed open circles indicate that the subjects received 10 micrograms potassium iodide (7.63 micrograms I^-)/kilogram body weight, either as carrier with the I^{131} or, in one patient, during the course of the uptake (vertical dotted line connecting the solid circle and the crossed open circle).

addition. As a rule, this increase was greater for those individuals with the higher rates of I^{131} accumulation, although some overlapping was evident (Fig. 1).

In those 8 individuals who received additional small doses of iodide there were further increases in the amounts of iodide collected by the thyroid. The iodine accumulation by the thyroid varied directly with the serum levels of iodide, providing the latter were relatively low (Figs. 2 and 3).

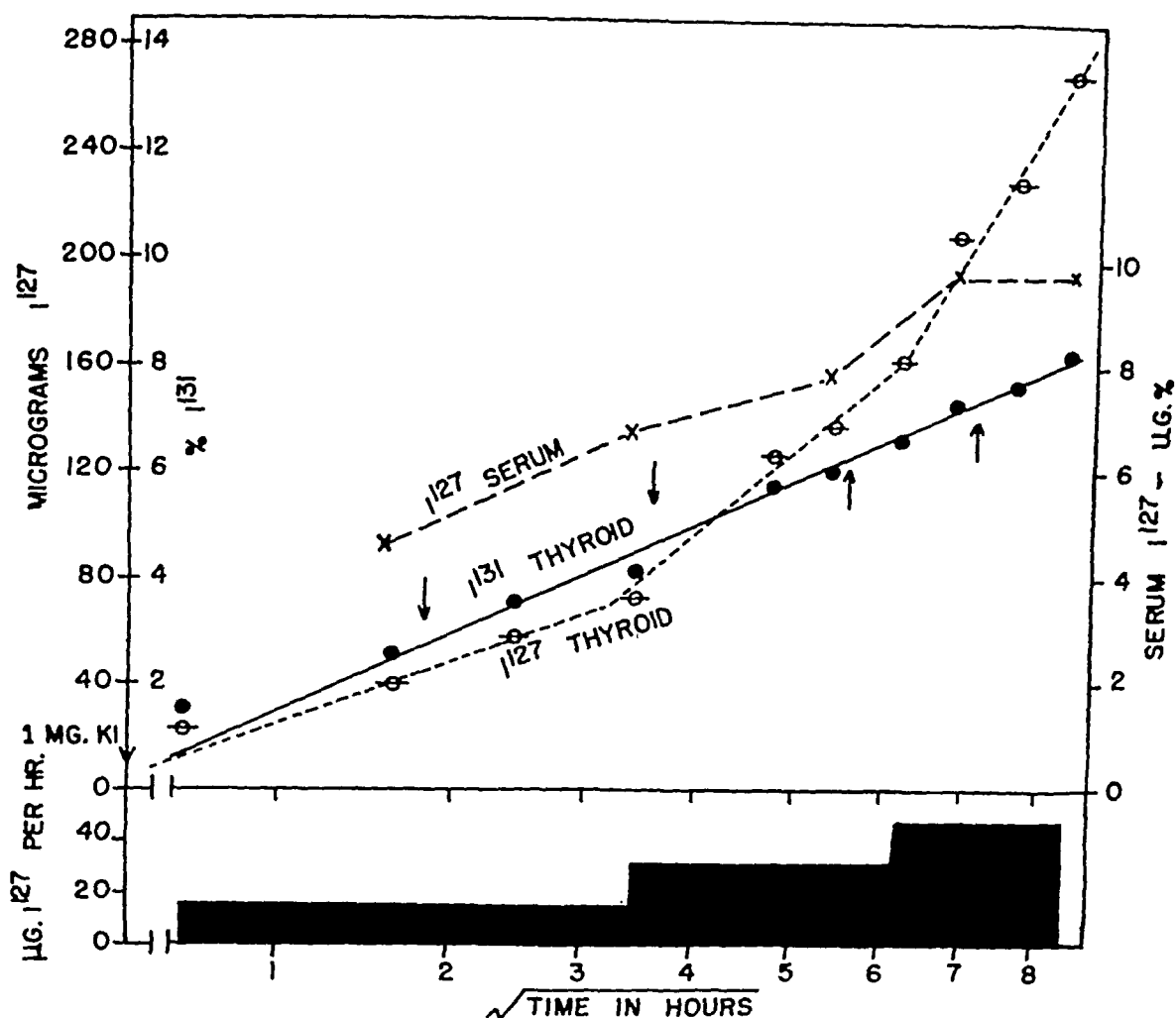


FIG. 3. The influence of several small doses of potassium iodide on the radioiodine and total iodine collection in a normal subject with a relatively inactive thyroid (I^{131} uptake 8 per cent in eight hours). The I^{131} accumulation is plotted against the "square root of time" to demonstrate the linear relationship. One milligram of potassium iodide was given as carrier and, during the test, additional doses of potassium iodide in half-milligram amounts, as indicated by the arrows. Although the serum level was raised to nearly 10 micrograms per cent by the total of 3 milligrams potassium iodide and the total iodine uptake was increased to 48 micrograms/hour, there was no inhibition of binding of iodine in this inactive gland.

ual as it appeared in a few instances, or always abrupt as it seemed in most subjects.

In the 13 thyrotoxic patients studied, there were large variations in the radioiodine collections and usually corresponding differences in total iodine accumulations. The average was 120 micrograms I^{127} /hour, and the range was from 44 to 265 micrograms/hour.

There was in these thyrotoxic glands a very large capacity for accumulating the iodide ion (5); even when organic binding of iodine was virtually completely inhibited by antithyroid drugs of the thiouracil type, such thy-

As serum iodide levels above 6 to 12 micrograms per cent were attained with larger amounts of iodide, the organic binding of iodine in the thyroid was halted (Fig. 4). The values for serum iodide with which inhibition of binding was detectable were inversely related to the rate of I^{131} collection. Thus, in subjects with very slow rates of collection, serum iodide values in the upper part of the range were necessary before inhibition occurred,

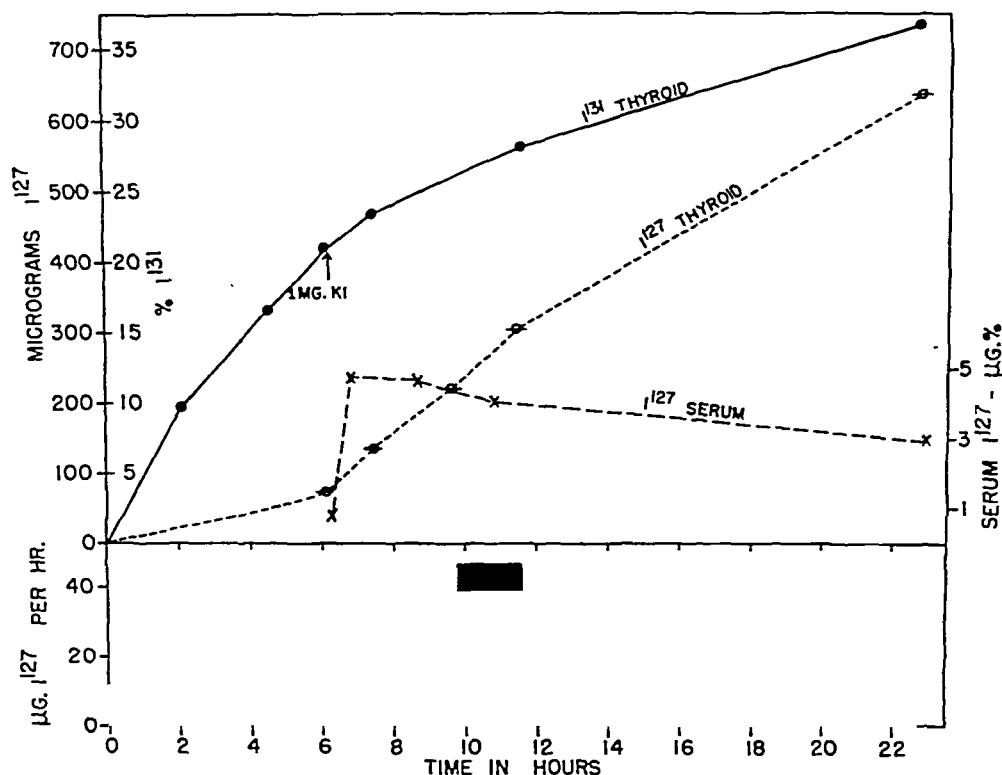


FIG. 2. The iodine utilization by the normal thyroid before and after administration of a small dose of iodide. The I^{131} accumulation curve is plotted against time to show the usual parabolic shape (solid line). The course of the I^{131} uptake was not changed by the dose of 1 milligram of potassium iodide which raised the serum level of iodide by 4 micrograms per cent (five times). However, the total (I^{127}) collection was increased approximately four times, from 11.7 micrograms/hour to 44.9 micrograms/hour, as shown by the dotted line and the bars.

whereas inhibition was attainable with levels around 6 micrograms per cent in others whose glands were very active.

Because of the appreciable amounts of iodine which could be accumulated and held as the iodide ion in the gland (see below), it was difficult to ascertain whether the cessation of organic binding due to iodide was grad-

"filled" before blocking was evident. Since the uptake pattern in hyperplastic thyroids could not be fitted to a straight line as in normals, deviations from this line could not be utilized as an index of inhibition.

However, the characteristic pattern of collection exhibited by the inhibited hyperplastic gland was recognizable (5) (Figs. 5 and 6). This con-

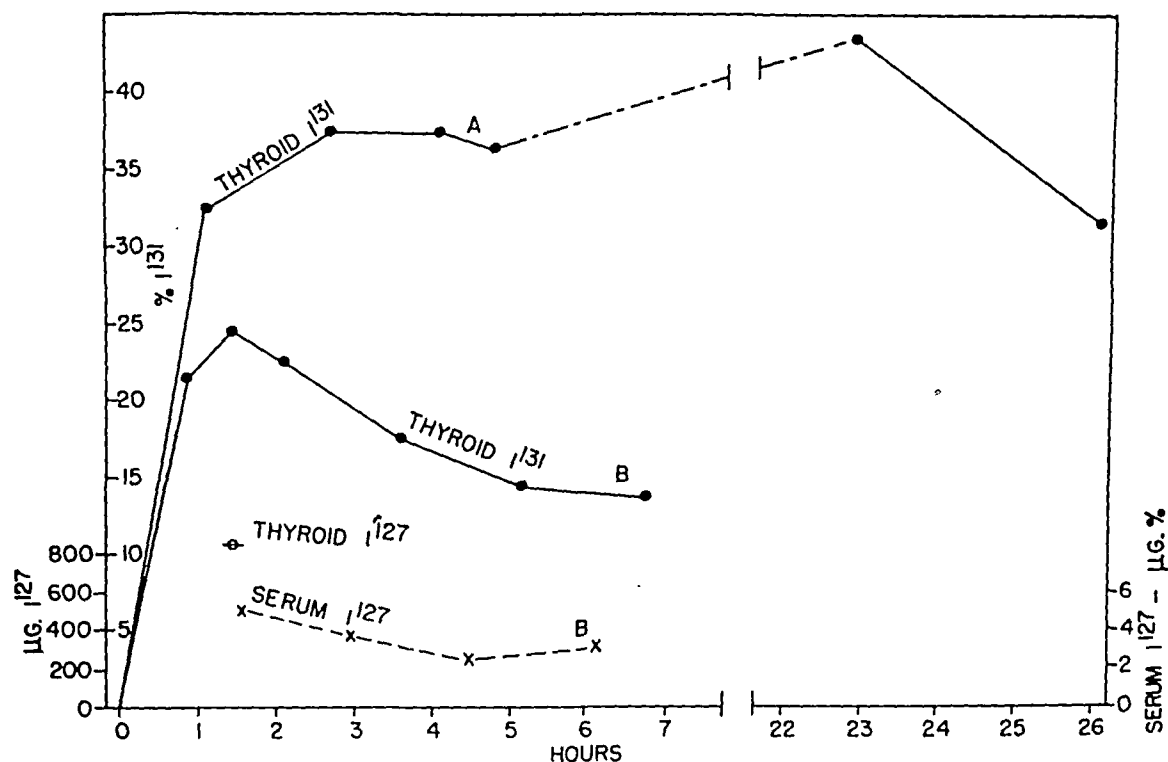


FIG. 5. The influence of small amounts of iodide on organic binding in 2 subjects with untreated thyrotoxicosis. Both received the iodide as carrier. In both, the characteristic shape of the curves denotes that the collection consisted largely of the iodide ion. In A (10 micrograms potassium iodide/kilogram body weight), there was some increase in I^{131} accumulation at the 23-hour interval, indicating that organic binding had occurred to some extent after this prolonged period. The decrease after thiocyanate administration (2 grams, given at the twenty-third hour) showed that an appreciable portion still remained as iodide ion, however. In B, (20 micrograms potassium iodide/kilogram body weight), with levels of serum iodide probably higher, the more rapid spontaneous decrease in the thyroid iodide I^{131} parallels the fall in serum iodide. The quantity of iodine in the thyroid in B, as shown by the crossed circle, was 827 micrograms during the second hour, mostly in the form of the iodide ion. No thiocyanate was given to this patient.

sisted of the rapid accumulation of 10 to 40 per cent of the I^{131} , which was usually at a maximum in two to three hours; following this there was a gradual or rapid fall as the radioactivity remaining in the gland in the form of freely diffusible iodide was diluted with serum iodide of progressively decreasing specific activity. The thiocyanate ion would cause discharge from the thyroid of all, or a large part of, that portion of iodine

roids could collect as much as 40 per cent of a dose of I^{131} as the iodide ion. It was repeatedly demonstrated that such large quantities as 15 milligrams of iodide (I^{127}) could thus be held in glands in which binding had been halted. In such instances the prompt detection of inhibition by iodide was difficult, particularly when the dose was given during the course of accumulation, because of the large size of this "iodide space," which must be

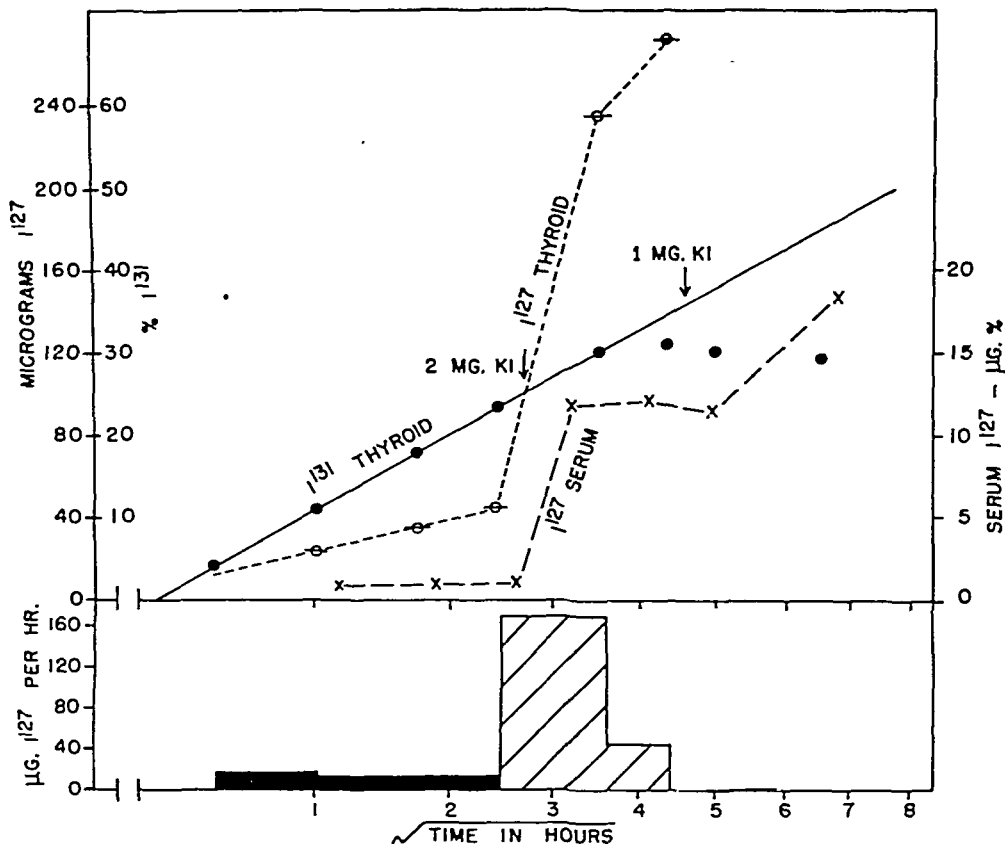
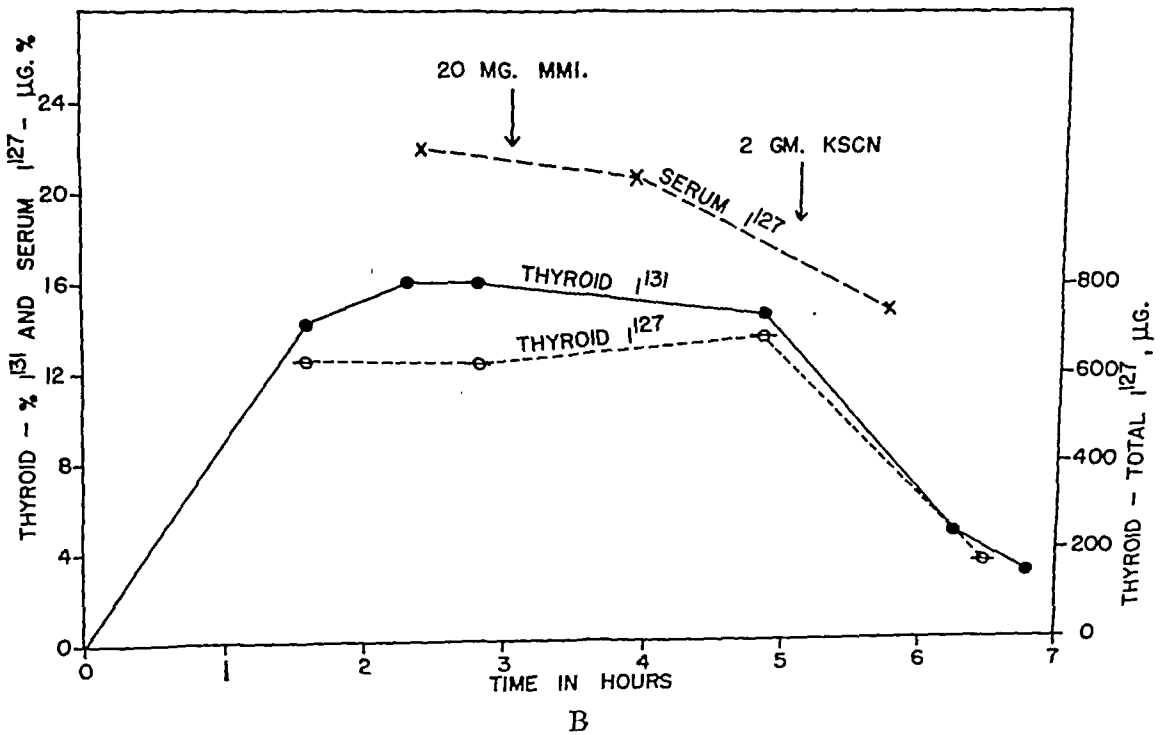
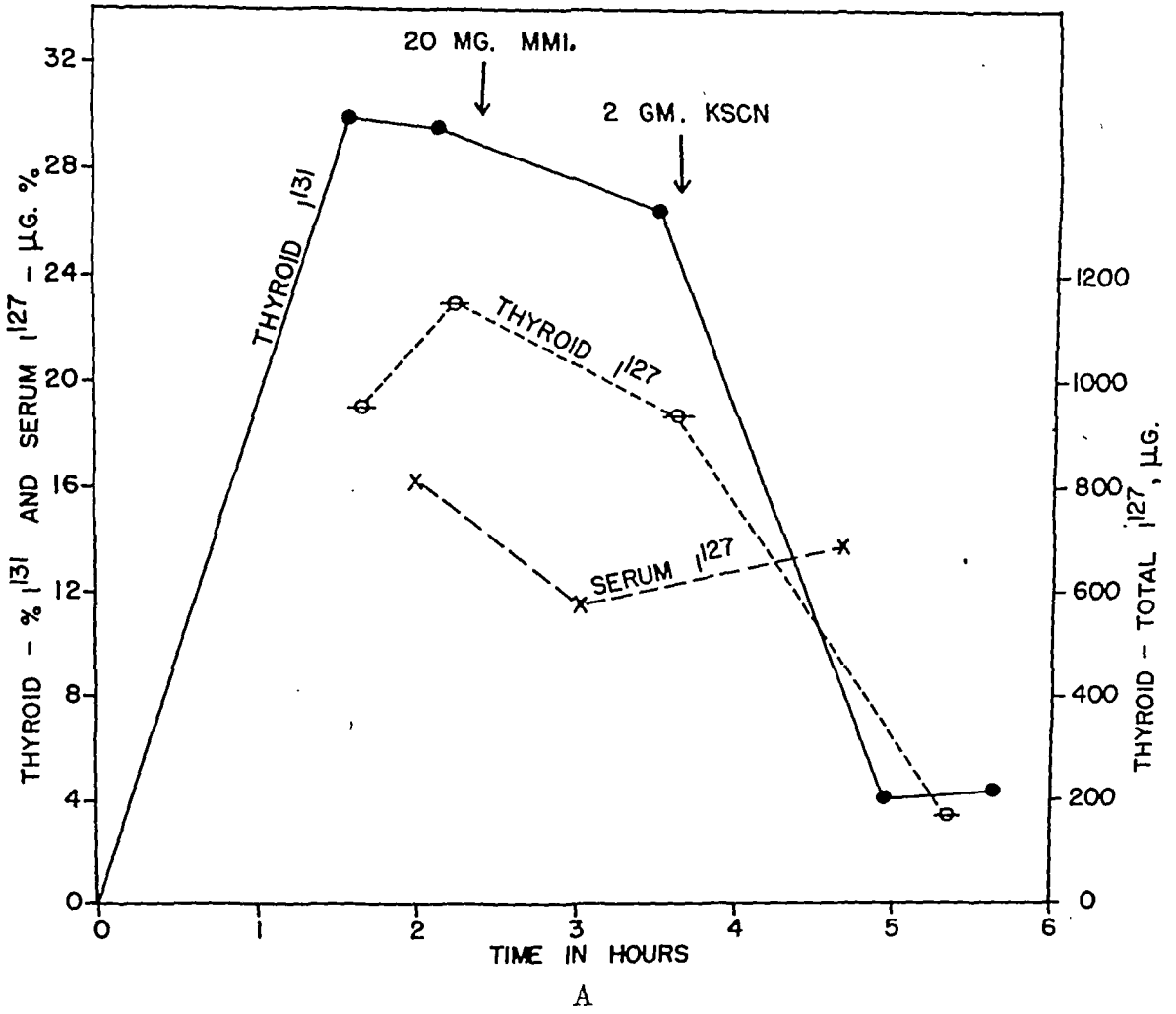


FIG. 4. The influence of a small but inhibitory quantity of iodide (2 milligrams potassium iodide) in a normal subject with a very active thyroid (I^{131} accumulation estimated by extrapolation to be 51 per cent at eight hours). The inhibition is shown by the cessation of accumulation of radioiodine soon after administration of the iodide. The large bars are hatched because the accumulations do not represent organically bound iodine solely. The larger hatched bar is spuriously high and represents a combination of : a) error in calculation (from continued uptake of high activity iodide before and during absorption of the added iodide); b) organic binding while the inhibitory levels were being achieved; and c) iodide ion collected after cessation of binding. The smaller hatched bar indicates an increment of iodide ion almost exclusively. That a part of the accumulated iodine was in the form of the freely diffusible iodide ion (unbound) is shown by the fall in I^{131} in the thyroid which occurred soon after the peak level was attained.



large in some thyroids (with slow rates of iodine turnover) before the I^{131} accumulation stopped (Fig. 3), whereas it was small in others (with relatively rapid uptakes), it could be inferred that cessation of I^{131} collection indicated actual inhibition of binding. Thus, the observations of Wolfe and Chaikoff in the rat were extended to man.

The observed values for serum iodide in patients who had not received iodine medication, 1 microgram per cent or less, agree well with those obtained by sensitive microchemical methods. Our results depend upon chemical analysis of iodine in the urine, which is a relatively easy chemical procedure. Since the iodine content is usually above 10 micrograms per cent, 10 to 20 cc. of urine can be readily analyzed. Hence it is felt that the results by this indirect method on serum are at least as accurate as those obtained by direct analyses.

From the observations reported here it may be deduced that in each individual the thyroid extracts a certain fraction of the iodide from the blood. This fraction remains constant, at least for a short time, with increasing levels of serum iodide until inhibition is attained. Presumably in euthyroid subjects the quantity of pituitary thyrotropin available is a most important factor in determining the size of the fraction of serum iodide extracted by the thyroid. The rate of I^{131} accumulation is an indication of the magnitude of the fraction, and, hence, of the potential capacity for hormone formation within the limits discussed in the next paragraph. However, in order to know how much iodine is being used for synthesis at any time, it is necessary to determine the specific activity of the metabolized iodine. Therefore the assumption that a rapid accumulation of radioiodine by the thyroid indicates a large total iodine utilization, such as accompanies thyrotoxicosis, may in certain instances be fallacious (Fig. 1). These same limitations are applicable when these functions are expressed as thyroid clearance of serum iodide.

Our data are consistent with the theory (1) that the concentration of iodide ion in the thyroid cells, rather than the serum iodide level, is the ultimate consideration governing the amount of iodide necessary to produce inhibition in a given instance. The thyroid cell iodide concentration is a function, not only of the serum iodide level, but also of the ability of the thyroid to

FIG. 6. The influence of larger amounts of iodide on organic binding of iodine in the thyroid glands of 2 additional patients with thyrotoxicosis. In both subjects 5 milligrams of potassium iodide were given by mouth (A, 40 minutes; B, 90 minutes) before the I^{131} . In both, the serum levels of iodide were in excess of the concentration required to produce inhibition of binding. The left vertical arrows ("MMI") indicate the administration of large doses of the potent antithyroid drug, 1-methyl-2-mercaptoimidazole; the reasons for this are discussed in the text. In each patient there was a small residual fraction of radioactivity in the gland following the action of thiocyanate. The interpretation of this finding is also undertaken in the text.

iodine in the thyroid gland occurred. In general, inhibition seemed to be determined by the level of iodide in the thyroid cell, which was a function both of the ability of the cell to concentrate iodide from the serum and the amount of iodide available in the serum. The serum iodide levels with which inhibition could be produced in thyrotoxic subjects were 5 micrograms per cent or less, whereas a higher value, between 6 and 12 micrograms per cent, was necessary to stop organic binding in the less hyperplastic glands of euthyroid individuals. These data are the results of acute experiments. No clue is provided to explain the relapses which occur during the course of treatment of Graves' disease with full doses of iodine. Alterations in the above reactions may occur after a period of iodination.

Acknowledgments

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THE ANTITHYROXINE ACTIVITY OF THYROXINE ANALOGS*†

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SINCE the discovery of the inhibitory action of para-aminobenzoic acid on the sulfonamides, the concept of antagonistic action between compounds closely related structurally has been applied to many fields of biologic activity, as reviewed recently by Woolley (1947 (1)). In 1946 (2) Woolley reported on the synthesis of a series of compounds which were antagonistic to thyroxine. Using as a criterion of such activity the ability of the compounds to protect tadpoles against lethal doses of thyroxine, he found that several ethers of N-acetyl-diiodothyrosine possessed the property of inhibiting the activity of thyroxine. Some of these compounds exhibited in addition a weak thyroxine-like effect.

To explore further the possibility of antagonizing the action of thyroxine by compounds related to it structurally, a series of substituted thyronine compounds were examined for their antithyroxine activity. These compounds were more closely related structurally to thyroxine than the compounds studied by Woolley. They were synthesized by Niemann (1941, 1941 b, 1944). Seven compounds were available for study,¹ namely:

1. Thyronine
2. 3'-Fluorothyronine
3. 3'-Fluoro-3,5-diiodothyronine
4. 3',5'-Difluoro-3,5-diiodothyronine
5. 3'-Fluoro-5'-iodo-3,5-diiodothyronine
6. 2',6'-Diiodothyronine
7. 3',5'-Diiodo-4 (4'-hydroxyphenoxy) 3,5-diiodo hippuric acid.

The structural configurations of these compounds are depicted in Figure 1.

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¹ Dr. Carl Niemann of the California Institute of Technology kindly supplied all the thyroxine analogs used in these experiments.

treated rats resulted in a quantitative inhibition of the hyperplasia of the thyroid gland caused by thiouracil, and these authors demonstrated the use of this phenomenon as a method for the assay of thyroid activity. Reineke, Mixner and Turner (1945 (9)) showed the close correlation between this method of studying thyroid activity and the standard metabolic method.

In the experiments reported here the thyroxine-like activity of each compound was determined by injecting the material into thiouracil-treated rats and comparing the thyroid gland weights with those of rats treated with thiouracil alone. Decrease in weight indicated thyroxine-like activity. A comparison of the decrease in thyroid weight with that attained by a standard dose of thyroxine (in this case 5.0 micrograms) indicated the relative potency of the two compounds. The ability of these compounds to antagonize thyroxine was determined by injecting each compound into thiouracil-treated rats which were receiving 5.0 micrograms of thyroxine. If the compound had the property of interfering with the action of thyroxine, the decrease in size caused by thyroxine would be inhibited and the hyperplasia usually brought about by thiouracil treatment would still be present.

The experiments were performed on male rats of the Sprague-Dawley strain weighing approximately 120 grams at the start of the experiment. They were kept in small cages with 2 rats in each cage. Food was Purina laboratory chow given *ad libitum*. The thiouracil was given as a 0.1 per cent solution in the drinking water. Each compound was tested on a group of 10 animals, unless otherwise indicated. In each series of experiments, the animals were treated for ten days with thiouracil, during which time they received daily injections of the compounds being tested. On the eleventh day the rats were killed with ether, the thyroid glands carefully dissected out and weighed on the torsion balance and the weight of the thyroid gland per 100 grams of body weight were calculated. For each group the mean and standard deviation were determined and, where indicated, significant differences between two means were determined by analysis of variance.

RESULTS

As a preliminary study, each of the 7 thyroxine analogs was tested in equimolecular doses for its thyroxine-like and antithyroxine activity, using 5.0 micrograms of thyroxine as a standard. This dose of thyroxine was found to be the minimum dose necessary to prevent completely the hyperplasia of the thyroid gland in rats treated for ten days with thiouracil. The experiments were performed in successive ten-day periods. Each new compound was tested by using 4 groups of rats. All the rats received thiouracil

The results of these experiments are summarized in Table 1. In examining the table, if one compares the results in groups 1 and 3 for any given compound, one can determine the presence of thyroxine-like activity. A significant decrease in thyroid weight in group 3 as compared with group 1 indicates that the compound has thyroxine-like activity because the thiouracil hyperplasia is suppressed. A comparison of group 3 with group 2 gives some indication of range of potency. A comparison of group 2 with group 4 gives information concerning the antithyroxine activity of any given compound. A significant increase in thyroid weight in group 4 over that in group 2 shows that the thyroxine activity is being inhibited.

Therefore it can be seen that the compound 2',6'-diiodothyronine definitely inhibited the activity of thyroxine. In two separate experiments this compound almost completely inhibited the suppressing effect of 5.0 micrograms of thyroxine on thiouracil hyperplasia. None of the other compounds exhibited any antithyroxine activity. In fact, several of them exhibited thyroxine-like activity, some to a marked degree.

To elucidate further the thyroxine-inhibiting activity of 2', 6'-diiodothyronine the compound was given to a group of normal rats, receiving no other treatment for a period of ten days, in a dose of 500 micrograms per day. At the end of this period the thyroid glands were weighed and also examined histologically. There was found to be no effect of this compound on thyroid weight (Table 2) or histologic appearance compared with control rats. Since it is generally believed that injected thyroxine probably becomes combined in some way within the body before it is active, it was postulated that perhaps 2',6'-diiodothyronine antagonizes the action of injected thyroxine by competing with it *in vivo* for some type of combination, whereas when the 2',6'-diiodothyronine is given to rats whose thyroid activity comes solely from the normally functioning thyroid gland, the combination has already occurred, and any competing analog would be ineffective.

TABLE 2

Treatment	No. of rats	Thyroid weight per 100 Gm. body weight (milligrams) Mean \pm S.D.
Control	5	7.90 \pm 0.76
2',6'-Diiodothyronine (500 micrograms per rat per day)	6	7.41 \pm 0.88

In order to put this hypothesis to the test, the ability of 2',6'-diiodothyronine to antagonize the action of thyroglobulin was determined. In thyro-

thyronine can antagonize the action of injected thyroglobulin as well as that of thyroxine, but the quantitative relations are certainly not clear cut.

To return to the other thyroxine analogs, examination of Table 1 demonstrates that neither thyronine nor 3'-fluorothyronine showed any thyroxine-like activity in a dose 150 times that of thyroxine. The other fluorine-substituted compounds all showed thyroxine-like activity, and in the dose given, *i.e.* 150 times the dose of thyroxine, were more effective than 5 micrograms of thyroxine. Therefore, further experiments were performed, using smaller doses of these compounds, to determine their relative potency. The data on these compounds are presented in Tables 4, 5 and 6. These tables show that the activity of 3'-fluoro-3, 5-diiodothyronine lies between one-twentieth and one-fortieth that of thyroxine, and that of 3',5'-difluoro-3,5-diiodothyronine is between one-thirtieth and one-sixtieth that of thyroxine. In the case of 3'-fluoro-5'-iodo-3, 5-diiodothyronine, it was found that this compound is at least one-third as active as thyroxine, and probably more so.

The final compound which was tested, namely 3',5'-diiodo-4 (4'-hydroxyphenoxy) 3-5-diiodohippuric acid was found to have a weak but definite thyroxine-like activity. A dose 150 times that of thyroxine showed an activity less than that of 5 micrograms of thyroxine, showing that this compound has less than 1/150 the activity of thyroxine.

DISCUSSION

The results of these experiments demonstrate that among the group of thyroxine analogs studied, one of them, namely 2',6'-diiodothyronine, had the ability to antagonize the action of thyroxine when the latter was injected into rats treated with thiouracil. Furthermore, it was also shown that this compound could inhibit the activity of injected thyroglobulin. It is interesting to note that 2',6'-diiodothyronine was the only compound in which the substitutions were in the 2',6' position on the phenyl ring. In all the other compounds, the iodine or fluorine substitutions were in 3,5 or 3',5' position on the phenyl ring. These compounds had either thyroxine-like activity or no activity at all; none of them showed any antithyroxine activity.

The mechanism of the inhibitory action of 2',6' diiodothyronine on thyroxine is not clear from these experiments. In the doses used, the antagonist had no demonstrable effect on the thyroid gland of normal untreated rats, suggesting that its action is not related to the production of thyroid hormone by the gland, such as is seen with thiouracil and related compounds. It is generally believed that thyroglobulin is broken down to simple polypeptides containing thyroxine before it circulates in the blood stream, and presumably before it acts on tissue cells. If it can be assumed that

TABLE 4. A COMPARISON OF THE POTENCY OF 3'-FLUORO-3,5-DIODOETHYRONINE WITH THYROXINE

Group	Treatment	Thyroid weight per 100 Gm. body weight (milligrams) Mean \pm S. D.
	All rats received 0.1% thiouracil to drink. In addition:	
1	0	16.03 \pm 1.80
2	Thyroxine—5 micrograms per day	7.66 \pm 1.55
3	3'-Fluoro-3,5-diiodothyronine 64.8 micrograms per day \approx 20 \times thyroxine	10.88 \pm 2.18
4	3'-Fluoro-3,5-diiodothyronine 140 microgram per day \approx 40 \times thyroxine	6.53 \pm 0.95

TABLE 5. A COMPARISON OF THE POTENCY OF 3',5'-DIFLUORO-3,5-DIODOETHYRONINE WITH THYROXINE*

Group	1	2	3
Dose of 3',5'-difluoro-3,5-diiodothyronine	Thiouracil alone	Thiouracil + 5 micrograms of thyroxine	Thiouracil + 3',5'-difluoro-3,5-diiodothyronine
220 micrograms per day \approx 60 \times thyroxine	18.96 \pm 3.40	5.55 \pm 0.85	6.11 \pm 1.13
108 micrograms per day \approx 30 \times thyroxine	16.03 \pm 1.79	7.66 \pm 1.55	10.73 \pm 2.56
72 micrograms per day \approx 20 \times thyroxine	16.03 \pm 1.79	7.66 \pm 1.55	13.82 \pm 2.11

* Each value represents the mean thyroid weight in milligrams per 100 Gm. body weight plus standard deviation for each group.

TABLE 6. A COMPARISON OF THE POTENCY OF 3'-FLUORO-5'-IODO-3,5-DIODOETHYRONINE WITH THYROXINE*

Group	1	2	3
Dose of 3'-fluoro-5'-iodo-3,5-diiodothyronine	Thiouracil alone	Thiouracil + 5 micrograms + thyroxine	Thiouracil + 3'-fluoro-5'-iodo-3,5-diiodothyronine
86.2 micrograms per day \approx 20 \times thyroxine	18.96 \pm 3.40	5.55 \pm 0.85	6.56 \pm 1.04
43.1 micrograms per day \approx 10 \times thyroxine	18.96 \pm 3.40	5.55 \pm 0.85	5.96 \pm 0.73
21.5 micrograms per day \approx 5 \times thyroxine	16.03 \pm 1.79	7.66 \pm 1.55	5.39 \pm 0.36
12.9 micrograms per day \approx 3 \times thyroxine	16.03 \pm 1.79	7.66 \pm 1.55	5.54 \pm 1.03

* Each value represents the mean thyroid weight in milligrams per 100 Gm. body weight plus standard deviation for each group.

in the second ring along with one fluorine atom, as in the compound 3'-fluoro-5'-iodo-3,5,-diiodothyronine. The activity of this compound closely approaches that of thyroxine. This demonstrates that the presence of only one iodine atom in the second ring results in a compound which is almost as active as thyroxine, which has two iodine atoms in the second ring. It would be of interest to know whether the fluorine atom in the 3' position is contributing anything to this activity. In view of the relative inactivity of the additional fluorine atoms in the compounds discussed above, this is doubtful.

A comparison of the activity of thyroxine and thyroglobulin as determined in these experiments, gives corroboration of the well-known fact that on the basis of thyroxine iodine, thyroglobulin is more active than

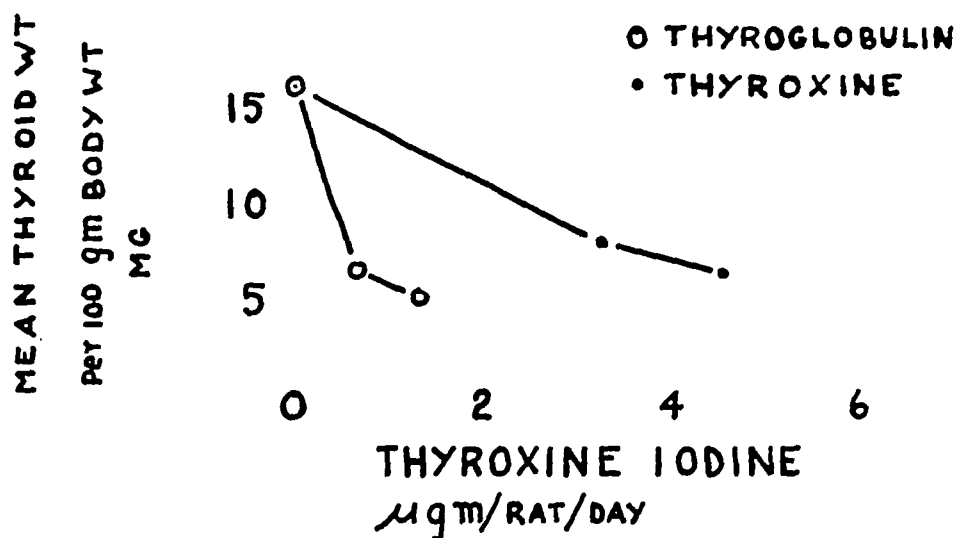


FIG. 2. The comparative effectiveness of thyroxine and thyroglobulin in inhibiting the thyroid gland weight in rats treated with thiouracil.

thyroxine. The thyroglobulin preparation had a value of 5.75 mg. of thyroxine iodine per 100 cc. Thyroxine has an iodine content of 65.4 per cent. The comparative effectiveness of these two compounds in suppressing thiouracil hyperplasia is plotted in Figure 2 on the basis of the thyroxine content of each compound. This graph shows conclusively that per unit of thyroxine iodine, thyroglobulin is much more effective than thyroxine. These results are significant, in that both compounds were injected subcutaneously, thus eliminating any differences on the basis of variations in absorption or destruction in the gastro-intestinal tract.

Harington in 1933 (14) showed that a peptide containing thyroxine obtained from thyroglobulin by enzymatic hydrolysis was more active than thyroxine alone and he postulated that "the activity of thyroxine can be enhanced by combination with other amino acids which occur in thyroglobulin." "... thyroxine is not to be regarded as itself the complete active

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CONFESSIONS OF AN ELDERLY THYROIDOLOGIST*

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A YEAR ago I had the honor of being your President. I was prevented from attending by unexpected and compulsory enrollment in an involuntary course in subjective medicine. This, I may say, was very instructive, but I deeply regretted that it conflicted with our Toronto meeting.

Dr. Crile has been so kind as to ask me to give today the address I had projected for a year ago. It is a belated Presidential address. The title, as you will perceive, is on the whimsical side. I do not really consider myself elderly, although I have been enjoying the thyroid for a relatively long time. Nor by "confessions" do I mean confessions of error only. As I read the dictionary, confessions may apply to confessions of success as well as of failure. I shall merely try to be well balanced and truthful. Finally, if anyone finds the term "thyroidology" offensive, I will plead that it is no worse than cardiology, gastro-enterology, or dozens of other ologies.

My first confession is that a gadget got me into the field of thyroidology. A passionate interest in biology originally got me into medicine, but why into the applied biology of medicine, rather than the fundamental science, I have not been able later to figure out. Probably a psychoanalysis would be necessary in order to do that.

However, I am quite clear that during my internship in 1912 I had every intention of going into the private practice of medicine as soon as I finished. My chief, the late David L. Edsall, decreed otherwise. He had become interested in the physiology of respiration and had had constructed what was then known as a Benedict Universal respiration apparatus. Having no time to use it himself, he got me a research fellowship and set me to work with it. After various explorations as to what it was good for, I settled down by 1914 to using it, primarily as a basal metabolimeter; and since alterations of basal metabolism had been shown by Magnus Levy and others to be greatest in thyroid disorders, the instrument drew me into that area.

My orientation at first was merely to use the basal metabolism as a quantitative index of thyroid function, and as such, an aid in diagnosis and

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her endocrines including the anterior pituitary and the adrenals. This is how we became conscious of pituitary myxedema, which is really a subspecies of Simmonds' disease. We have seen many cases since, and therefore must have been missing them before. However, there is no reason to miss them, if in all cases of myxedema other endocrine hypofunctions besides hypothyroidism are carefully looked for.

Since the entry of the pituitary our investigative work has been focused more and more on the activities and interrelations of thyrotropin and thyroid hormone, on the sensitivity of their end organs, and on agents which affect the balance between them. In 1929 a long series of studies with W. T. Salter began on the relation between chemical structure and physiologic activity of thyroid gland derivatives, and there is a confession concerned in that. Salter had brought back from Harington's laboratory in London, a sample of thyroxine polypeptide which he wished to have assayed in human myxedema. This was done, and it led to further studies. Among other questions, that of the effect of optical activity of thyroxine was put to the test. Harington supplied us with what were taken to be pure samples of dextro-rotary and of laevo-rotary thyroxine. When assayed in cases of myxedema these preparations had equal calorogenic activity. This seemed queer, because in general in nature laevo-rotary molecules are more active physiologically than their dextro-rotary isomers. However, we had to accept what we observed until more evidence was secured. In 1947, the experiment was repeated with new lots of material received from Harington. This time the laevo-rotary form was ten times more active than the dextro-. The interpretation accepted was that, in the first preparations, the separation of the two optically active isomers had been imperfect. It has become abundantly evident that thyroxine is the business part of the thyroid hormone molecule. Yet it also appears that when given by mouth to man, the total physiologic activity of a thyroid preparation is related more nearly to its total organic iodine than to its thyroxine iodine. I will confess that this paradox still puzzles us.

As I look back over my whole experience in the thyroid field, which now covers a period of thirty-five years, I find the story falls into fairly definite chapters. With regard to Graves' disease, there was first the pre-iodine era; then the post-iodine, and finally, the era of radioactive iodine and antithyroid drugs. All of these agents we promptly made the subject of study as soon as they were introduced.

When radioiodine came in, I predicted that it would prove an invaluable tool for research but I was dubious about its ultimate usefulness in therapy. The first part of the prediction has come true; the latter remains in doubt, not because the agent isn't effective but because we don't yet know

HASHIMOTO'S DISEASE*

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IN the past few years we have seen an increasing number of goiters which are classified as Hashimoto's type of chronic thyroiditis. We want to bring this to your attention, along with several other observations we have made in the 28 cases of the disease seen from 1935 to the present time. All but 2 of these cases were encountered since January, 1943.

In 1912 Hashimoto (1) described "struma lymphomatosa," which differed from other forms of chronic thyroiditis and especially from Riedel's (2, 3, 4) "eisenharte struma." Since that time there has been considerable controversy as to whether they actually are different processes.

In 1922 Ewing (5) studied 4 cases and concluded that Hashimoto had described the early, and Riedel the late stages of the same process. Others who take this same view include Boyden, Coller and Brugher (6), Perman and Wahlgren (7), Williamson and Pearse (8), Heyd (9), Eisen (10), Womach (11), Reist (12), and Shaw and Smith (13). In 1931 Graham and McCullagh (14) gave impressive support to the belief that these are two separate types of thyroiditis. McClintock (15), and Scarello (16), have shown that the tissue of the thyroid did not change from the Hashimoto type toward the Riedel type, but remained the same, judging from biopsy specimens taken two and thirteen years later respectively. Lee (17), Joll (18), Heineke (19), Moore and Lloyd (20), Lee and McGrath (21), Poer, Davison and Bishop (22), McSwain and Moore (23), and Decourcy (24), join the ranks of the separate entity followers. DeCourcy (25), furthermore, suggests that it is possible to have both processes in the same gland simultaneously.

CLINICAL MATERIAL

Struma lymphomatosa is not a respecter of geographic or social boundaries, and is found very predominantly among females. None of our cases was in a male and we have noted only 8 reports in the literature in which Hashimoto's disease was found in the male.

The average age of the patients as given in the literature by various authors varies from 43.8 years (26) to 57.6 years (18), the youngest being

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et al. (37), who claim that they have one patient who shows no hypothyroidism after five years. Others who agree about x-ray treatment are Means (38), Schilling (39) and George Crile, Jr. (40), but Boyden, Coller and Brugher (6), and Marshall, Meissner and Smith (41) do not use it because they feel that it may further reduce the amount of thyroid secretion. McSwain and Moore (23), however, state that the incidence of hypothyroidism after x-ray therapy is not as high as after operative procedures.

In 1934 Polowe (42) reported a case of Hashimoto's disease in which the B.M.R. was plus 43 per cent and it was discussed at the meeting of the American Association for the Study of Goiter in 1940. Crane (53) and later Polowe (42) and Womach (11) proposed that hyperthyroidism was the first sign of Hashimoto's disease. In view of the microscopic picture and the fact that our patients were of a younger average age than those previously reported and had higher basal metabolic rates, we feel that we have been dealing with many early cases. We are inclined to agree that in the early stages of Hashimoto's disease there is a slight hyperthyroidism, followed by euthyroidism and then hypothyroidism.

SUMMARY

Twenty-eight cases of Hashimoto's disease seen since 1935 are reported. Twenty-six of these cases were seen since 1943.

The average age of these patients was less than that previously reported in the literature, and the preoperative basal metabolism was higher.

Following total thyroidectomy, mild myxedema developed in 61.5 per cent of the patients; whereas, following subtotal thyroidectomy, it developed in only 6.7 per cent of the patients. Hypothyroidism occurred in 89.3 per cent of the total number of cases.

A review of the theories concerning the etiology of the disease is presented.

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roidectomy was advised: 1) merely to remove the lumps, and 2) because of attacks of auricular fibrillation.

February 17, 1947: Bilateral subtotal thyroidectomy was done under ethylene and oxygen anesthesia without an intratracheal catheter. The anesthesia was begun at 8:30 and terminated at 9:40 a.m. There was no difficulty with the anesthesia and the blood pressure, pulse rate and respiratory rate were within the usual normal limits throughout the operation. The patient had an ordinary bilateral multiple nodular goiter.

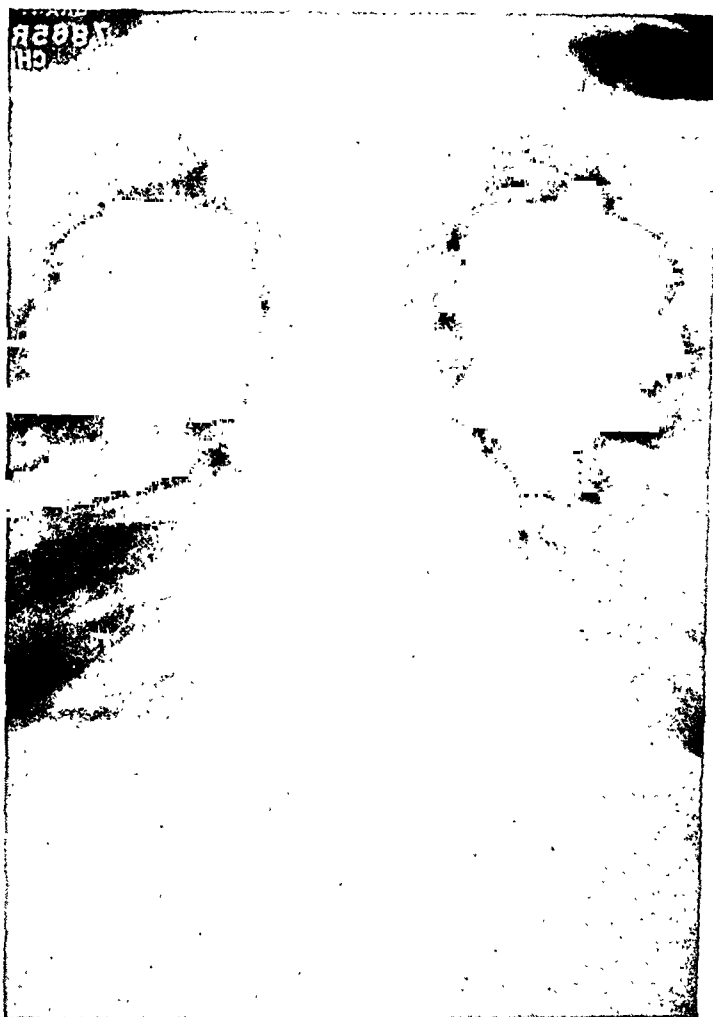


FIG. 1. Case 1. Massive bilateral pneumothorax with mediastinal and subcutaneous emphysema, five hours after thyroidectomy.

The removed lobes weighed 114 grams. The operative procedure was uneventful. The inferior thyroid artery was ligated proximally on the right side and the recurrent laryngeal nerve was visualized on that side. A small Penrose drain was left in the wound.

The patient was returned to his room awake and in good condition. At 1 p.m. he complained of severe pain in the left side of his chest and difficulty in breathing. The respiratory rate rose to 28 per minute and the pulse rate from 90 to 130. He was immediately placed in an oxygen tent. It was thought that he had a massive collapse of the lung.

of carbon dioxide from the lungs. There was no subsequent evidence of a parathyroid deficiency.

It was noted that the respirations consisted of a long expiratory phase and a very short, almost instantaneous, inspiratory phase. From the character of the breathing it was suspected that he had a tension pneumothorax. On examination of the chest it seemed that the left side was more resonant and that the breath sounds were much less distinct. A roentgenogram (Fig. 2) taken at 10:30 a.m., one hour after operation, re-



FIG. 2. Case 2. Almost complete left pneumothorax, one hour after thyroidectomy.

vealed a large left pneumothorax and mediastinal and subcutaneous emphysema. The subcutaneous emphysema was never marked. It could not be seen on inspection but could be felt by palpation above each clavicle.

A needle was inserted anteriorly through the chest wall in the second interspace. A considerable quantity of air was withdrawn but it was not measured and the needle then was connected to a water-seal bottle. The patient's condition improved rather slowly. It was several hours before he regained a reasonable degree of consciousness. Cyanosis and dyspnea persisted in a lesser degree but sufficient to necessitate continuous

Kjaergaard (40) demonstrated such a valve vesicle in one autopsy and possibly in a second but most pathologists search for them in vain. It must be admitted, however, that the openings through which air seeps from the mediastinum into the pleural cavity are also not discernible to the post-mortem pathologist. But much higher pressures are required to rupture the normal visceral pleura than the thin mediastinal pleura. It is undoubtedly possible for PT to result from this cause but it does not seem possible that the PT thus produced would result in mediastinal and subcutaneous emphysema. Although air passes rather easily from the mediastinum into the pleural cavity it cannot be forced under ordinary pressures from the pleural cavity into the mediastinum. In Adams' case (6) the patient had asthma plus emphysematous lungs as proven by preoperative x-ray examination. This is the only case reported in the literature in which there was any abnormality of the lung itself. His patient had a right tension PT, apparently without mediastinal or subcutaneous emphysema. No autopsy was performed. That the PT arose from a ruptured vesicle is unquestionably possible. Yet Peterson (41) reported a death from ME and PT in asthma and collected 7 from the literature, in all of which there was associated ME. The presence of ME is in itself presumptive evidence that in these cases the air passed from the mediastinum into the pleural cavity rather than the reverse.

Macklin and Macklin (42) have recently made an exhaustive review of ME and PT with an interpretation of the clinical literature in the light of laboratory experiments. From it one can obtain practically all the useful information that there is on the subject. It is their contention that the syndrome of PT, ME and subcutaneous emphysema begins as a pulmonary interstitial emphysema due to the rupture of intrapulmonary vesicles. As a result of local or general hyperinflation, air ruptures through the vesicles based on the sheaths of small branches of the pulmonary vessels. It makes its way along the vessel sheaths to the mediastinum. It may then make its way upward into the neck or downward along the aorta and esophagus. It may rupture the mediastinal wall producing pneumothorax. Once the leakage has begun, the pressure necessary to continue the leak need not be so high as that initiating the rupture. In some patients, the air builds up sufficient pressure within the lung itself to block off the pulmonary circulation and even bronchoalveolar ventilation. In some, it builds up dangerous pressure within the mediastinum. In some, the air passes upward into the neck with relief of mediastinal pressure. In some, it ruptures into one or both pleural cavities with or without tension pneumothorax. The path it will take is not always the same and is not predictable. This theory will satisfactorily explain the history and findings of all the reported cases of ME and PT following thyroidectomy. No other one will.

ME in 12 of 18 cases and are of the opinion that the air enters through the neck. Neffson (38) found PT and ME in 17 out of 126 tracheotomies. Forbes *et al.* (12) found ME after tracheotomy in 30 (41 per cent) of 71 patients checked by x-ray examination and PT in 12 (15 per cent) of 82 patients. There were two deaths from this cause alone in a total of 120 tracheotomies. This high incidence of ME and PT following tracheotomy would tend to confirm the theory that the air reaches the pleural cavity by way of the neck. Yet Figi (39) reported 200 tracheotomies and did not mention the complication at all. His patients differed from those of the other authors in two respects: a) they were mostly adults, whereas the others were children, and b) the tracheotomies were not as rule performed as emergency procedures for severe obstruction, as in the case of the others. Analysis by Neffson (38) and Forbes (12) indicated that ME and PT increase in incidence with the severity of obstructive symptoms. Most of the cases of ME and PT reported following thyroidectomy were in patients also exhibiting evidence of respiratory obstruction during the course of the operation. Seidl (10), much as did Goldberg (37), injected air into the dog's mediastinum through a glass tube. He found that with 100 cc. of air there was no change; at 300 cc., no change; but with 600 to 800 cc., with pressures of plus 22 to plus 26 cm. of water, there was a sudden drop of pressure to minus 8 to 10 cm. when air entered the pleural cavity. In a patient with an intrathoracic goiter he found a mediastinal pressure of 0 to minus 3 cm. of water in ordinary breathing and from minus 8 and minus 10 cm. to plus 2 and plus 4 cm. on deep breathing. In three patients with an outspoken intrathoracic goiter he found no evidence of ME by x-ray examination four hours postoperatively. He could not obtain in animals any pressure rise in the mediastinum by the simple sucking in of air. Although he admits the possibility of air being sucked into the mediastinum, he cannot conceive of sufficient pressure being built up to cause its rupture into the pleural cavity. In his opinion, if air entered the mediastinum by this route it should be absorbed or even come out of the wound or through the drainage opening. Indeed, the surgical treatment of severe ME is an incision above the sternal notch to permit the exit of air. In the second case reported here, very large quantities of air were withdrawn from the chest over a period of three days during which time the wound, although it contained a small latex rubber (Penrose) drain, was sealed over and certainly could not have given ingress to such a large volume of air. Similar circumstances led Adams (6) to a similar conclusion. Obviously, this hypothesis for the explanation of the condition following neck operations could not be applied to abdominal and other operative procedures.

The rupture of an emphysematous bleb with a valvular flap on the surface of the lung is a commonly accepted explanation for spontaneous PT.

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incidence of complications was noted than in patients prepared with Lugol's solution. The most striking result that has been obtained is the reduction of operative mortality. In the twenty-year period preceding 1943, the operative mortality for all patients with hyperthyroidism was approximately 1 per cent, and in a selected group of patients, the thyrocardiacs, it was approximately 3 per cent.

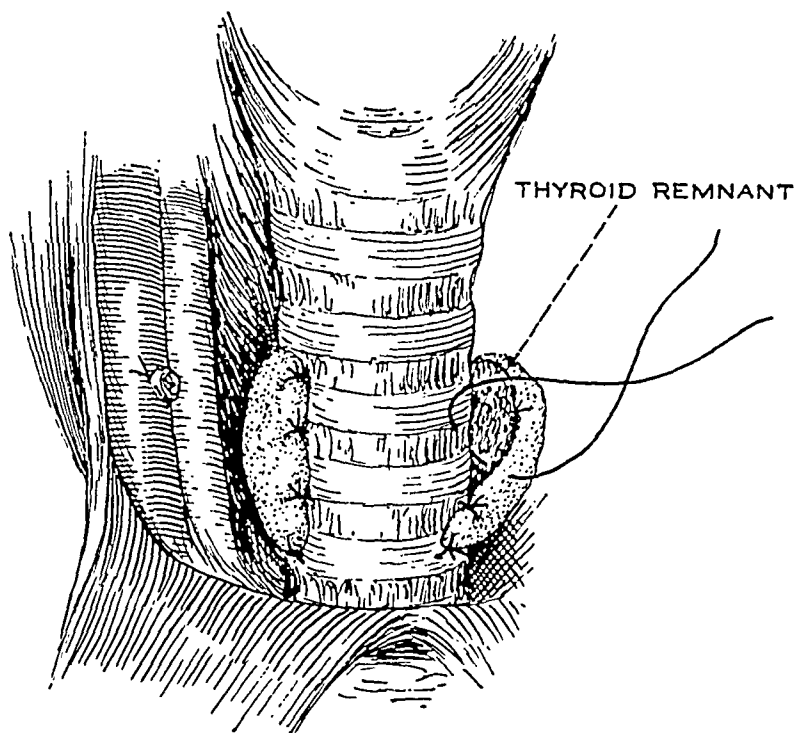


FIG. 2. A small remnant remains after radical subtotal thyroidectomy. The anterior edge of the remnants is sutured by interrupted sutures to the fascia of the trachea. (From *Surgical Clinics of North America*, June 1949. Published with the permission of W. B. Saunders Company, Philadelphia.)

Operative Procedure

All thyroidectomies were carried out under general anesthesia, utilizing nitrous oxide-oxygen-ether or ethylene-oxygen-ether anesthesia. The majority of patients had the anesthetic given through an endotracheal tube. Cyclopropane has not been used because of its effect on the heart, particularly as evidenced by changes in rhythm. No patients have been operated on under local anesthesia.

The technic of subtotal thyroidectomy will not be described. The operation is carried out routinely by the division of the prethyroid muscles and with a wide lateral dissection and exposure. All four thyroid arteries are ligated, the superior thyroid vessels being ligated and divided and the inferior thyroid arteries ligated in continuity (Fig. 1). Two or more parathy-

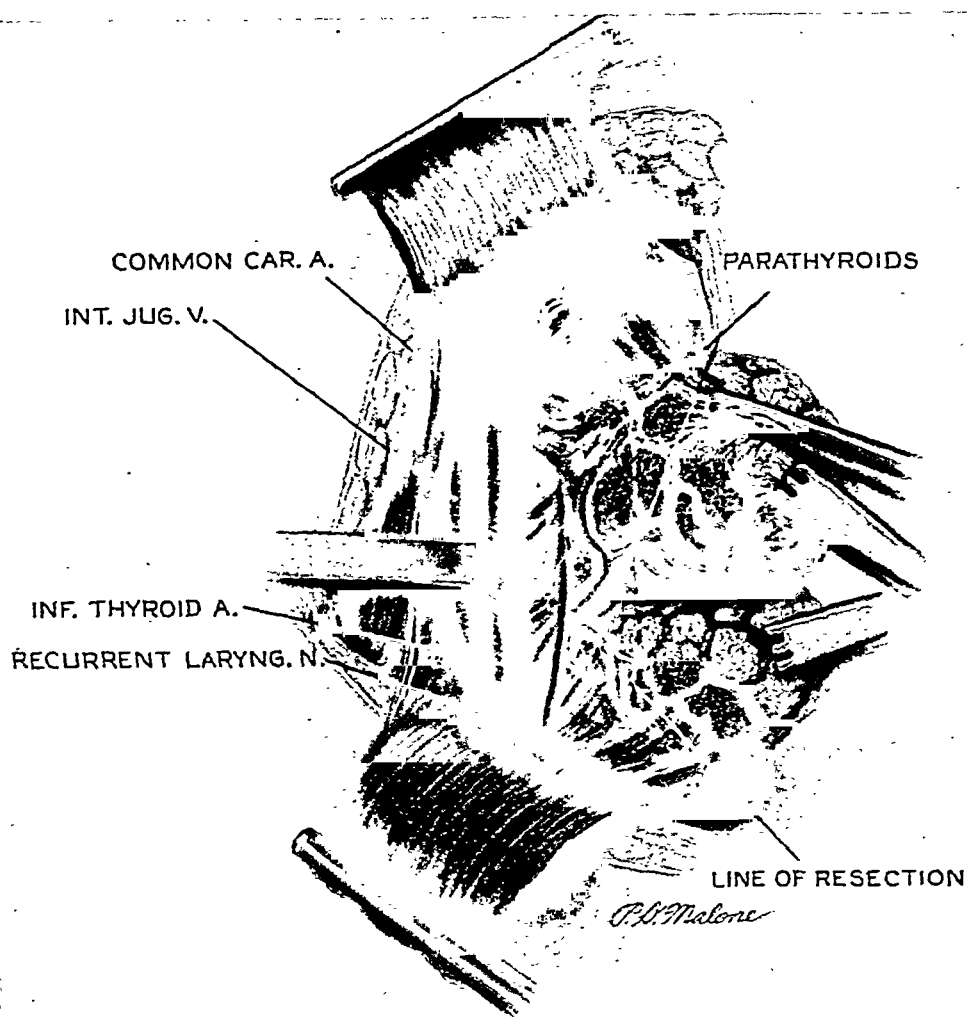


FIG. 1. The superior thyroid vessels have been doubly ligated and divided. The superior and inferior parathyroids have been identified. The recurrent laryngeal nerve is demonstrated for its entire cervical course. The inferior thyroid artery will then be ligated in continuity. (From Surgical Clinics of North America, June 1949. Published with the permission of W. B. Saunders Company, Philadelphia.)

vascularity and to be very friable. The appearance of the gland was not unlike that observed in patients before the use of iodine. Most of these technical difficulties have been overcome by causing involution of the gland by the administration of iodine during the latter part of the pre-operative period but it still remains necessary to exercise the greatest care in carrying out the procedure, in order to avoid the complications that may follow thyroidectomy. This became evident soon after the employment of the antithyroid drugs when, as reported in an earlier review, a higher

of all four thyroid arteries but may still occasionally be encountered in spite of this technical precaution. Most of the bleeding encountered is from the veins of the skin flap or the anterior jugular veins but at times the inferior thyroid veins may not be adequately secured. In 1,000 consecutive patients undergoing thyroidectomy it was necessary to open the incision in 27, or 2.7 per cent. It is our practice to open any wound that becomes puffy or ecchymotic, since there is always the possibility of these conditions being followed by some degree of respiratory obstruction. In any doubtful case it seems advisable to open the flap and make certain that all bleeding is controlled.

Respiratory obstruction requiring tracheotomy

Thirteen patients out of the group of 1,000 had tracheotomy performed either at the time of operation or soon afterward, usually within forty-eight hours. Any patient with signs of respiratory obstruction has immediate indirect laryngeal examination and if the airway is encroached upon by edema or there is limited motion of the vocal cords, tracheotomy is performed at once. It should be emphasized that tracheotomy should be performed in any doubtful case, since a mild degree of respiratory obstruction may rapidly become a complete obstruction with little further warning. In the 2 fatalities that occurred from this cause, the tracheotomy was performed four and six hours respectively after the first symptoms occurred, yet this proved to be too long an interval. Nurses, interns, residents and staff physicians must all be aware of this possibility and the necessity for immediate action in order to avoid the rare fatality that may result.

In this group of 13 patients in whom a tracheotomy was performed, were 3 patients who had preoperative myxedema produced by the use of the antithyroid drugs. This occurred early in our experience and it is now appreciated that respiratory obstruction is very likely to develop in any patient with myxedema under these circumstances. Each patient routinely has a metabolism test on the day that he is scheduled for operation. If the metabolism is found to be depressed below normal, the operation is cancelled, blood is taken for determination of (fasting) cholesterol, and the patient is discharged from the hospital. Iodine medication is continued but no further antithyroid drugs are given for a period of two to four weeks. In our more recent experience, after recognition of this possibility, respiratory obstruction from this cause has not occurred.

Parathyroid tetany

The incidence of postoperative parathyroid insufficiency has increased considerably since the use of the antithyroid drugs. There is no definite evidence to support the view that the antithyroid drugs affect the parathy-

In the present series of 1,000 patients, unilateral nerve injury was found in 10, or 1.0 per cent. In addition to this small number, there were other patients who had temporary immobility of the cord as observed by indirect laryngeal inspection. We do not believe this is related to exposure of the nerve but rather to edema as a result of thyroidectomy.

TABLE 1. SURGICAL TREATMENT OF HYPERTHYROIDISM. SUMMARY—1000 PATIENTS

	<i>Per cent</i>	
Hemorrhage	2.7	8.5
Tracheotomy	1.3	
Postoperative hypothyroidism	4.5	
Tetany	1.5	5.1
Nerve injury	1.0	
Recurrence	2.4	
Mortality	0.2	

In Table 1, I have made a summary of the complications and sequelae of thyroidectomy in 1,000 consecutive patients prepared with antithyroid drugs and iodine. If one includes the patients who have postoperative bleeding and those in whom a tracheotomy was necessary, 4 per cent of the patients have technical complications which cause difficulty during the immediate postoperative period but which do not interfere subsequently with obtaining a good result. About an equivalent number, or 4.5 per cent, require the administration of desiccated thyroid after operation, making a total of 8.5 per cent. Operative mortality and recurrent laryngeal nerve injuries are found to have a very low incidence. It is very essential that every effort be made to reduce the incidence of parathyroid insufficiency and recurrence of the hyperthyroidism, since these two groups constitute 4 per cent of unsatisfactory results.

CONCLUSIONS

Surgery is the most satisfactory method of treatment for most patients with hyperthyroidism. It should be employed, with few exceptions, in patients with nodular goiter with hyperthyroidism.

Subtotal thyroidectomy can be carried out with a low operative mortality which has been further lowered to 0.2 per cent by the utilization of the antithyroid drugs in the preparation of patients for operation.

When antithyroid drugs are used in the preparation of patients for surgery, larger portions of the thyroid gland should be removed than after preparation with iodine.

Following subtotal thyroidectomy satisfactory results are obtained in 95 per cent of patients.

TABLE 1. INCIDENCE OF CARCINOMA IN NODULAR GOITER (INCLUDING TOXIC, NONTOXIC, AND CARCINOMA) AT THE ILLINOIS RESEARCH HOSPITAL, 1936-1948.

Type of goiter	1936-1944		1944-1948		1936-1948	
	No. of cases	Per cent carcinoma	No. of cases	Per cent carcinoma	No. of cases	Per cent carcinoma
Toxic nodular	330	1.2	48	0	378	1.0
Solitary	(71)	0.0	(17)	0	(88)	0.0
Multinodular	(259)	1.6	(31)	0	(290)	1.4
Nontoxic nodular	192	17.1	93	17.2	285	17.15
Solitary	(92)	24.0	(51)	(25.5)	(143)	(24.4)
Multinodular	(100)	(11.0)	(42)	(7.1)	(142)	(9.8)
<i>Total</i>	522	7.2	141	11.3	663	8.0

TABLE 2. INCIDENCE OF CARCINOMA IN NODULAR GOITER AS OBTAINED FROM NUMEROUS REPORTS IN THE LITERATURE

Author	Number of patients with nodular goiter (toxic and nontoxic)	Per cent carcinoma in nodular goiter (toxic and nontoxic)	Number of patients with nodular nontoxic goiter	Per cent carcinoma in nodular nontoxic goiter	Per cent carcinoma in solitary nontoxic nodular goiter
Brenzner & McKnight (6) Charlotte, N. C. 1940	2,324	4			
Horn & assoc. (7) Philadelphia, Pa. 1947	1,135	6.3	637	9.8	
Crile Cleveland, Ohio 1948	537	5.6	274	10.9	24.5
Ward San Francisco, Calif. 1947	3,539	4.8			15.6
Cole & assoc. Chicago, Ill. 1948	663	8.0	285	17.1	24.4

of 24.5 per cent carcinoma in solitary goiter, although Ward records an incidence of only 15.6 per cent (Table 2).

Although it has long been known that goiter is a geographic disease, that fact seems to be forgotten, particularly when the incidence of carcinoma is being considered. There is ample statistical evidence to prove the point regarding incidence of goiter. For example, Rogers and associates (4) recently noted that in 544,918 patients admitted to the Boston City Hospital, the Massachusetts General Hospital and to Johns Hopkins Hospital over a period of several years, only 0.59 per cent were admitted to the hospital because of a goiter. We made a survey of 68,573 admissions to the Illinois Research Hospital over a period of eleven years and found that 1.7 per cent of the patients coming to the hospital came because of a goiter. This incidence of goiter in our patients is almost exactly three times greater than the incidence in the patients reported in the series by Rogers and associates.

We should also like to comment on the failure of autopsy figures to reflect correctly the incidence of carcinoma of the thyroid. For example, over a period of the last four years our Department of Pathology has had only 2 autopsies for carcinoma of the thyroid. However, during this same period we know that 11 of our patients with carcinoma of the thyroid have died. None of these 11 was autopsied in our hospital. The 2 who were observed came from our previous series. We did not refer any of our patients with inoperable carcinoma of the thyroid to the hospital in the moribund state for autopsy, largely because beds are so scarce that there is not room even for patients with acute conditions. Since we already knew the diagnosis in our patients with metastases, and knew there was nothing more we could do for them, we did not refer them to the hospital. Most of them died at home, although we assume that perhaps a few might have died in other hospitals.

It might be said that our high incidence of carcinoma in toxic nodular goiter is related to too much liberality in making the diagnosis. Unfortunately, our follow-up for our first series for 1936-1944 was poor. However, when we had evidence that carcinoma of the thyroid appeared to be present in such great frequency, we decided to follow all subsequent patients very carefully. We are now able to report a 100 per cent follow-up in patients with carcinoma of the thyroid since 1944 to date. Of 16 patients with carcinoma of the thyroid observed between 1944 and a few months ago, we know that 11 are already dead. About half of these were inoperable when we first saw them. A twelfth patient has metastasis; a short time ago we resected the sternoclavicular joint for a metastasis which decreased in size under radioactive iodine, but did not disappear. We assume that this patient has other evidence of metastasis, but knowing that the survival time in carcinoma of the thyroid is extremely variable, it is possible that

WHAT THYROID NODULES ARE TO BE FEARED?*†

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IN 1947 two articles appeared from the Eastern Seaboard of the United States minimizing the importance of carcinoma of the thyroid (1) (2). In the first, this disease was described as a rare cause of death. This assumption was based on the low incidence of carcinoma of the thyroid found at autopsy at three hospitals in Boston. The author was unable to explain why the incidence found by him was so low in contrast to reports of others and suggested that the pathologic criteria used in making the diagnosis were responsible.

The second article reported a study of both clinical and autopsy material at three hospitals. The incidence of carcinoma of the thyroid in the periods studied was found to be less than that reported elsewhere, and virtually no greater than the mortality rate of prophylactic surgery in nodular goiter at two of the hospitals. Since there was an undesirable complication rate in addition, strong doubt was expressed regarding the advisability of such prophylactic surgery.

These reports are at variance with the experience of others. Cancer of the thyroid exists along the Eastern Seaboard as well as in the goitrous districts of the United States. Since both of the above papers were based upon statistics gathered in part from the Massachusetts General Hospital, and since the experience and considered opinion of the Thyroid Clinic of this hospital differs from the conclusions reached in both papers, it seems proper to set before you the reasons leading to our opinion and the policy of management of carcinoma of the thyroid at the Massachusetts General Hospital.

Incidence of carcinoma of thyroid at the Massachusetts General Hospital

The incidence of carcinoma of the thyroid at the M.G.H. has been determined over the twelve-year period from January 1937 through Decem-

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neoplasia as a localized disease. Such recognition is relatively easy when the disease is in the form of a single nodule and the remainder of the gland is normal. It is less easy when the whole of the gland is nodular and recognition of neoplasia depends upon differentiating the character of one nodular area apart from the whole. The local characteristics of hardness, irregularity and adherence are helpful adjuncts in indicating malignancy but may be misleading owing to the presence of inflammation.

The hope of adequate therapy is increased the earlier the lesion is recognized. Although spread of the carcinoma into the neighboring tissue and lymphatics generally indicates a primary lesion of considerable duration, some thyroid cancers, particularly the papillary type, may metastasize early into the lymphatic channels. Search for lymph node enlargement, an indication of metastasis, is always indicated even when the suspected primary lesion is still small and not locally invasive.

It is not within the scope of this paper to give in detail an account of the spread of carcinoma of the thyroid into the lymphatic channels. I should like to point out, however, a lymph node or group of lymph nodes, the Delphian node, which has not received attention elsewhere but which we have found of great usefulness in identifying the early spread of cancer of the thyroid into the lymph nodes (5).

The Delphian node, named after the oracle of Delphi, lies in the midline of the neck just above the upper border of the thyroid isthmus and in front of the middle cricothyroid ligament. Anterior to it lies the first cervical fascia and the skin. Normally the lymph node is not palpable but if it is enlarged and firm from invasion of metastatic carcinoma, it is readily palpable for two reasons. First, there is no platysma overlying it because the platysma muscle does not come to the midline, there being a gap between the muscles on the two sides. Second, it can be felt against the firm cricoid cartilage or cricothyroid ligament beneath it and can be rolled under the fingers. There is difficulty in feeling lymph nodes elsewhere in the neck during the early phase of their enlargement from metastatic cancer because they are easily displaced or depressed against the soft tissues around and behind them. It is not until enlargement is more advanced that they can be felt against the jugular vein, for example.

The location of the Delphian lymph node is shown in Figure 1. The lymph node is almost always normally present. At 152 operations at which this lymph node was specifically looked for it was found in all except nine cases, or 94 per cent. In 64 per cent of the 152 cases but one lymph node was found. In 21 per cent two nodes were found: in 6 per cent, three nodes; and in 3 per cent, four or five nodes. When the nodes are multiple they are clustered together. The higher number of nodes was found generally when disease was present in the thyroid associated with lymph node enlargement, that is, carcinoma, thyroiditis or Graves' disease hyperplasia.

WHEN IS MALIGNANT GOITER MALIGNANT?*

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IN any discussion of malignant goiter there is always a question as to just what the author means by malignant goiter. In the literature for the past twenty-five years, and especially in the discussions which have taken place before this Association and other scientific organizations, there is considerable controversy as to whether this or that lesion should be included in the group under discussion, whether this or that pathologic criterion is definitive in the determination of malignancy, whether pathologic or microscopic findings alone can determine malignancy or whether the diagnosis can be reached only on a clinico-pathologic basis. My thesis is that only by clinical study, painstaking pathologic investigation and by a long personal follow-up can it be determined whether some of the more controversial cases are malignant or not. It is with the hope of answering some of these questions that the following study of 178 cases is presented.

Group I

First of all there are cases in which there is no doubt of the presence of malignancy. This is the group of highly malignant tumors, undifferentiated microscopically, rapidly growing, invasive locally and prone to metastatic involvement of the lungs or other distant regions. These tumors seem insensitive to radiation therapy, prone to postoperative recurrence and uniformly prove rapidly fatal. Incidentally, death from the tumor or its metastases is the final incontrovertible proof of malignancy. A few examples of this group will serve to illustrate the point (Fig. 1). There were 48 (27 per cent) of these patients in my series and they are all dead of the disease, with an average length of life of seven months from the time of diagnosis. Nobody will question malignancy here. These tumors prove their nature in rapid order by killing their hosts. Incidentally, there is one pathologic finding of grave prognostic import in these cases, namely, polymorphonuclear infiltration as seen in some of the microscopic sections (Fig. 1, C and E). So much for the noncontroversial group.

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Fig. 4. Roentgenogram of J. R. E. (case 42) taken at the age of 8 years 10 months. There has been no change in this picture in nineteen years. Insert shows section of tumor. ($\times 80$).

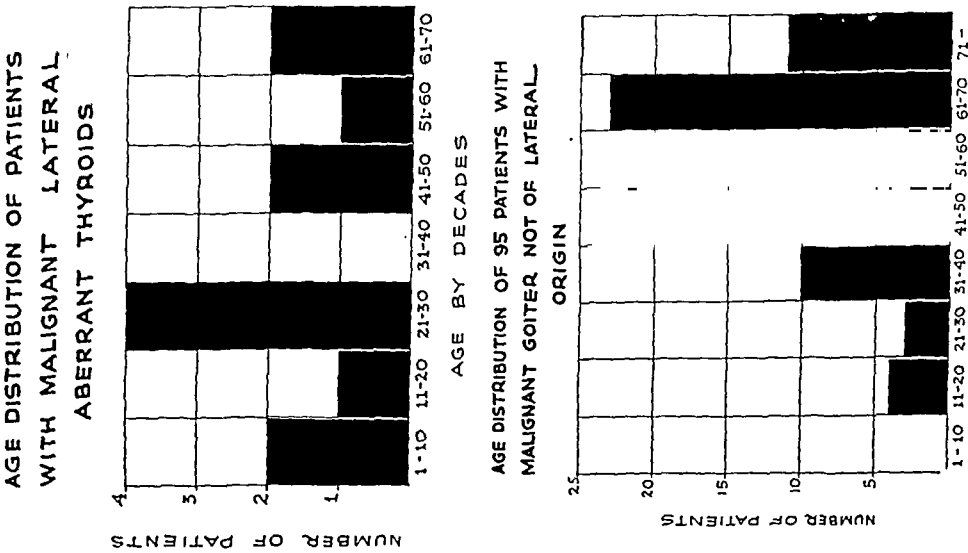


Fig. 3. Graphs illustrating early age at which cancer of aberrant thyroid tissue occurs as contrasted with that of midline origin.

toms. Their incidence in a degree warranting further antithyroid treatment or operation is reported by various authorities as being from 2.3 per cent (3) to 17.5 per cent (4, 5, 6). In this series, 32 patients (12.3 per cent) had secondary procedures performed as the second, third, or fourth thyroidectomy.

If we acknowledge these recurrences, then we must also acknowledge that subtotal thyroidectomy is not an entirely satisfactory treatment for diffuse toxic goiter. The same may be said for medical management, including radiation, since it is generally conceded that these measures are associated with even higher recurrence rates following cessation of the treatment. This appears to be true of propylthiouracil as well as of drugs less recently introduced. Microscopically the thyroid tissue of patients treated with thiouracil and its derivatives displays a cytologic structure not unlike that observed in the untreated, highly toxic hyperthyroid patient. Available drugs are not capable of restoring the thyroid gland to normal and of so maintaining it.

Obviously, then, we cannot fulfill the first mentioned hypothetical requirement for the permanent relief of hyperthyroidism and there remains only the second stated idea of treatment, that is, the total ablation of all abnormal thyroid secretion. This can be accomplished by total thyroidectomy. If such a procedure can be carried out with a reasonable morbidity and mortality rate, then it should be worthy of consideration. We believe today, as we did in 1939, that we can perform total thyroidectomy with a morbidity and a mortality rate comparing favorably with those attendant upon subtotal operative procedures.

Total thyroidectomy was abandoned by Kocher and others in favor of subtotal procedures because of the complications and sequelae, particularly the postoperative myxedema. The operation was replaced by resection not because of any inherent superiority of the latter procedure, but because of the lack of knowledge and the lack of means to combat such conditions as 1) postoperative athyrea, 2) parathyroid tetany, and 3) recurrent laryngeal nerve injury. These circumstances initiated the trend toward subtotal thyroidectomy which has persisted to this day. It has been our observation that the incidence of the mechanical complications and sequelae in this series compares favorably with the incidence following subtotal thyroidectomy. We shall briefly consider our experience with these once prohibitive factors.

Postoperative follow-up

In this series of 280 patients, 269 (96.1 per cent) have been studied subsequent to operation, as noted in Table 1. Of this group, 78.9 per cent have been followed two years or longer. Only 11 patients (3.9 per cent) have not

TOTAL THYROIDECTOMY IN THE MANAGEMENT OF DIFFUSE TOXIC GOITER*

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IT is the purpose of this paper to present our experience with total thyroidectomy in the management of diffuse toxic goiter. This report reviews a series of 280 patients operated on by one-stage procedures during the fourteen-year period from 1935 to 1949. We feel that total thyroidectomy offers a rational surgical approach to the problem and that it may eliminate certain inadequacies of subtotal thyroidectomy.

In 1939, Scott (1) presented before this Association a preliminary report on 71 total thyroidectomies, wherein he outlined the reasons which justified the procedure. We believe these statements to be just as applicable today as then and wish to recall the following points:

Many years ago, Henry Plummer (2) pointed out that the manifestations of exophthalmic goiter appeared to be due to a qualitatively abnormal secretion rather than to a mere excess of a normal thyroid secretion. Apparently little attention has been paid to this early premise. However, we still do not know the cause of diffuse toxic goiter and today, we, as did Plummer, can only speculate as to the true etiology of the disease.

If Plummer's premise is assumed to be correct, then it follows that so long as the thyroid gland or any remnant thereof remains *in situ*, it may secrete an abnormal substance capable of producing any or all of the manifestations of hyperthyroidism in varying degrees of severity. It should also follow that these manifestations can be abolished in one of two ways: 1) either the thyroid tissue producing the abnormal secretion must be converted to normal thyroid tissue producing a normal secretion, or 2) there must be complete ablation of all of the abnormal thyroid secretion.

We believe that Plummer's hypothesis merits reconsideration and that it may explain the persistent symptoms observed following subtotal thyroidectomy, since such a procedure leaves a remnant of abnormal thyroid tissue *in situ*.

It is a fact well known to all of us that persistent manifestations do occur following subtotal operations. They are noted as tachycardia, nervous excitability, fatigue, weight loss, exophthalmos, and numerous other symp-

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maintained following total thyroidectomy by the proper administration of desiccated thyroid.

Parathyroid tetany

The majority of parathyroid disturbances in our series have been temporary in nature and easily controlled. These temporary manifestations apparently are due to a circulatory disturbance occurring during and after the operation and they subside in a few days. On the basis of this observation, we now routinely administer 100 units of parathyroid extract daily during the first five postoperative days. Since this innovation four years ago, no such temporary disturbance has been noted. Prior to this, 12 instances of transient tetany occurred. All of these patients were relieved by treatment before dismissal from the hospital.

TABLE 3. PARATHYROID TETANY

Type of tetany	Number of patients	Percentage of total
Transient	12	4.3
Chronic*	3	1.1

* Only 1 patient (0.36%) requires dihydrotachysterol (A T 10).

It will be noted in Table 3 that chronic tetany occurred in 3 patients (1.1 per cent). In all three, the disease is controlled by treatment. Two of these patients are maintained on oral calcium alone. It is questionable whether these 2 patients should be classified under chronic tetany since their symptoms are very mild, occur infrequently, and are controlled by oral calcium. In only 1 instance has dihydrotachysterol (A T 10, Winthrop) been required in the daily dosage of .625 milligram to maintain normal blood calcium levels. In no instance has there been a fatality from this complication and no chronic tetany has occurred since 1940.

Recurrent laryngeal nerve injury

We have noted a temporary disturbance of laryngeal function on 9 occasions. These are listed in Table 4 as transient nerve injuries, since the symptoms subsided in a few days and subsequent examinations have re-

TABLE 4. RECURRENT LARYNGEAL NERVE INJURY

I. Transient nerve injury
9 unilateral
II. Permanent nerve injury*
2 bilateral
2 unilateral

* Incidence of permanent injury—1.4%.

TABLE I

Patient	Age	Sex	Type of goiter and date of operation	Symptoms* ("S") after operation; persistent or recurrent	Aggravating factors†	Previous treatment and results	Presenting signs at beginning of antithyroid drug therapy	Antithyroid drug therapy	Results
1. R.D.J.	46	F	Toxic diffuse 1926—Ligation 1935—Subtotal thyroidectomy	Diffuse enlargement. Persistent until thyroidectomy. "S": 2, 4, 5, 6, 7, 8, 11.	None	Lugol's solution for 9 years. No improvement.	BMR plus 2%; no palpable thyroid; no weight change; cholesterol, 217 mg.%; "S": 2, 4, 5, 6, 7, 8, 11; secondary anemia.	Thiouracil 0.3 Gm. daily for 4½ months; iron for secondary anemia.	BMR minus 4%; thyroid not palpable; no weight change; anemia improved; "S": 4, 5, 6, 7, 11.
2. M.B.	44	F	Toxic diffuse 1929—Subtotal thyroidectomy followed by cord paralysis	No palpable thyroid; BMR not known. "S": 1a, 2, 3, 4, 6.	None	Lugol's solution intermittently for 16 years. No improvement.	BMR minus 14%; no palpable thyroid; no weight change; "S": 1a, 2, 3, 4, 6.	Thiouracil 0.2 Gm. daily for 10 months; propylthiouracil 0.050 Gm. daily for 4 months.	BMR minus 9%; thyroid not palpable; no weight change; "S": 1a, 2, 3, 4, 6.
3. M.Ba.	38	F	Toxic diffuse 1928—Subtotal thyroidectomy	No palpable thyroid; BMR not known. "S": 1a, 2, 4, 9, 10, 11.	Menopause	Lugol's solution intermittently for 17 years.	BMR minus 6%; no palpable thyroid; no weight change; "S": 1a, 2, 4, 9, 10, 11.	Propylthiouracil 0.150 Gm. daily for 18 months.	BMR minus 3%; no palpable thyroid; no weight change; "S": 1a, 2, 4, 9, 10, 11.
4. I.K.	43	F	Toxic diffuse 1934—Subtotal thyroidectomy 1937—Subtotal thyroidectomy	Persistent. "S": 1, 1a, 2, 4, 6, 8. BMR minus 13%; no palpable thyroid; no weight change; progressive exophthalmos; "S": 1, 1a, 2, 4, 6, 8.	Menopause	Lugol's solution; x-ray therapy, and bilateral tarsorrhaphy.	BMR minus 25%; no palpable thyroid; no weight change; marked exophthalmos; "S": 1, 1a, 2, 4, 6, 8.	Thyroid substance and stilbestrol. No antithyroid drug given because of progressive exophthalmos.	BMR minus 2%; no palpable thyroid; no weight change; "S": 1, 1a, 2, 4, 6, 8.
5. H.C.	36	F	Toxic diffuse 1942—Subtotal thyroidectomy	BMR plus 5%; slight thyroid enlargement; no weight change. Persistent. "S": 1, 1a, 2, 3, 4, 6, 7, 8, 9, 10, 12.	Psychic trauma	Lugol's solution and x-ray therapy to pituitary.	BMR minus 5%; no palpable thyroid; no weight change; "S": 1, 1a, 2, 3, 4, 6, 7, 8, 9, 10, 12.	Thiouracil 0.4 Gm. daily for 15 months.	BMR minus 1%; no palpable thyroid; weight loss 3 Kg.; "S": 1, 1a, 2, 3, 4, 6, 7, 8, 9, 10, 12.
6. C.M.	50	F	Toxic diffuse 1928—Subtotal thyroidectomy	BMR not known. No palpable thyroid. 1942: pernicious anemia; diabetes mellitus. Recurrent hyperthyroidism; "S": 1a, 2, 3, 4, 6, 9, 12.	Psychic trauma	Treatment with liver extract, diet, insulin.	BMR minus 5%; no palpable thyroid; weight loss; "S": 1a, 2, 3, 4, 6, 9, 12.	Thiouracil 0.3 Gm. daily for 14 months; propylthiouracil 0.100 Gm. daily for 3 months. No antithyroid drugs for 18 months.	BMR plus 32 and 29%; no palpable thyroid; weight loss 18 Kg.; anemia improved; diabetes poorly controlled; "S": 1a, 2, 3, 4, 6, 9, 12.

* Symptoms ("S") include: 1. Exophthalmos; 1a. Lid-lag; 2. Palpitation; 3. Tremor; 4. Nervousness; 5. Exertional dyspnea; 6. Sweating or heat intolerance; 7. Lassitude; 8. Headache; 9. Insomnia; 10. Phobias; 11. Chest pain; 12. Diarrhea.

† Aggravating factors include: Intercurrent infection, psychic trauma, pregnancy, menopause.

TABLE 2

Patient	Age	Sex	Type of goiter and date of operation	Symptoms* ("S") after operation; persistent or recurrent	Aggravating factors†	Previous treatment and results	Presenting signs at beginning of antithyroid drug therapy	Antithyroid drug therapy	Results
7. B.K.	52	F	Toxic diffuse 1940—Subtotal thyroidectomy	BMR not known; no weight loss. <i>Recurrent</i> after 5 years. "S": 1, 1a, 2, 3, 4, 6, 9, 10.	Menopause	Lugol's solution for 2 months without improvement.	BMR plus 3%; weight loss 9 Kg.; thyroid bilaterally enlarged; "S": 1, 1a, 2, 3, 4, 6, 9, 10.	Thiouracil 0.4 Gm. daily for 1 month; propylthiouracil 0.150 Gm. daily for 16 months; estrogens; no treatment for 12 months.	BMR minus 9%; weight gain 6 Kg.; thyroid not palpable; "S": 1, 1a, 4, 9, 10.
8. Z.B.	49	F	Toxic diffuse 1922—Subtotal thyroidectomy	BMR not known; no weight loss; bilateral thyroid enlargement. <i>Recurrent</i> after 19 years; "S": 1, 1a, 2, 3, 4, 5, 6, 8, 9, 10.	Menopause	Lugol's solution for 1 year with good control; no control since.	BMR plus 5%; no palpable thyroid; no weight loss; "S": 1, 1a, 2, 3, 4, 5, 6, 8, 9, 10.	Propylthiouracil 0.100 Gm. daily for 6 weeks <i>followed by drug fever</i> ; Lugol's solution for 21 months; estrogenic therapy; still under treatment.	BMR minus 18%; weight loss 2 Kg.; thyroid not palpable; "S": 1, 1a, 4, 8, 9, 10.
9. T.B.	36	F	Toxic diffuse 1939—Subtotal thyroidectomy	BMR not known; weight loss 5 Kg.; bilateral thyroid enlargement. <i>Recurrent</i> after 9 years. "S": 1, 1a, 2, 3, 4, 6, 9.	Pregnancy	None	BMR plus 57%; weight loss 5 Kg.; bilateral thyroid enlargement—R>L; "S": 1a, 2, 3, 4, 6, 9.	Propylthiouracil 0.200 Gm. daily for 9 months; still under treatment.	BMR minus 11%; weight gain 3 Kg.; thyroid smaller; "S": 1a.
10. B.L.	31	F	Toxic diffuse 1938—Hemithyroidectomy 1938—Hemithyroidectomy 1941—Subtotal-thyroidectomy	BMR not known; weight loss not known; no palpable thyroid. <i>Persistent</i> . "S": 1, 1a, 2, 3, 4, 5, 6, 8, 9.	None	Lugol's solution without relief; 10 x-ray treatments Feb.-Oct. 1944.	BMR plus 23%; thyroid not palpable; no weight change; "S": 1, 1a, 2, 3, 4, 5, 6, 8, 9.	Thiouracil 0.3 Gm. daily for 9 months; propylthiouracil 0.150 Gm. daily for 16 months. No treatment for 16 months.	BMR minus 16%; weight gain 3 Kg.; thyroid not palpable; "S": 1, 1a.
11. J.B.	48	F	Toxic diffuse 1943—Subtotal thyroidectomy	BMR plus 35% before operation; BMR plus 52% after operation; weight loss not known; bilateral thyroid enlargement; progressive exophthalmos. <i>Persistent</i> . "S": 1, 1a, 2, 3, 4, 5, 6, 8, 9, 10.	Menopause	Lugol's solution without relief; (iodide rash).	BMR plus 25%; Rt. lobe enlarged; weight loss unknown; "S": 1, 1a, 2, 3, 4, 5, 6, 8, 9, 10.	Thiouracil 0.4 Gm. daily for 3 months; propylthiouracil 0.200 Gm. daily for 19 months; estrogens; no medication for 8 months.	BMR minus 29%; weight gain 9 Kg.; thyroid not palpable; "S": 1, 1a.
12. C.J.	29	F	Toxic diffuse 1943—Subtotal thyroidectomy 1944—Subtotal thyroidectomy	BMR not known; weight loss unknown. <i>Persistent</i> . "S": 1a, 2, 3, 4, 6, 8, 9.	None	Lugol's solution without relief.	BMR plus 30%; no palpable thyroid; weight loss unknown; "S": 1a, 2, 3, 4, 6, 8, 9.	Thiouracil 0.6 Gm. daily for 4 months; no treatment for 2 years.	BMR minus 10%; weight gain 1 Kg.; thyroid not palpable; "S": 1a.

* and †: See corresponding footnotes to Table 1

TABLE 2—(Continued)

Patient	Age	Sex	Type of goiter and date of operation	Symptoms* ("S") after operation; persistent or recurrent	Aggravating factors†	Previous treatment and results	Presenting signs at beginning of antithyroid drug therapy	Antithyroid drug therapy	Results
19. G.D.	31	F	Toxic diffuse 1943—Subtotal thyroidectomy	BMR plus 48% before operation; no palpable thyroid; no weight change. <i>Persistent.</i> "S": 1a, 2, 3, 4, 6, 8, 9.	None	Lugol's solution for 6 months without relief.	BMR plus 27%; bilateral thyroid enlargement; no weight change; "S": 1a, 2, 3, 4, 6, 8, 9.	Thiouracil 0.8 Gm. daily for 4 months. No treatment for 8 months.	BMR minus 2%; no palpable thyroid; weight gain 1 Kg.; "S": 1a.
20. C.S.	36	F	Toxic diffuse 1926—Subtotal thyroidectomy	BMR unknown; no palpable thyroid; weight loss unknown. <i>Recurrent</i> after 19 years. "S": 1, 1a, 2, 3, 4, 6, 8, 9.	Ruptured appendix with generalized peritonitis.	Developed symptoms after recovery from peritonitis.	BMR plus 23%; no palpable thyroid; weight loss unknown; "S": 1a, 2, 3, 4, 6, 9.	Thiouracil 0.4 Gm. daily for 1 year; no treatment for 8 months.	BMR minus 4%; no palpable thyroid; weight gain 22 Kg.; "S": 1a.
21. H.P.	35	F	Toxic diffuse 1940—2-stage subtotal thyroidectomy	BMR unknown; weight loss unknown; thyroid enlarged again soon after operation. <i>Persistent.</i> "S": 1, 1a, 2, 3, 4, 6, 8, 9, 10.	Psychic trauma	Lugol's solution without relief.	BMR plus 24%; weight loss unknown; thyroid enlargement; Rt. lobe and isthmus; "S": 1, 1a, 2, 3, 4, 6, 8, 9, 10.	Propylthiouracil 0.100 Gm. daily for 30 months; psychotherapy for 14 months; still under both forms of treatment.	BMR minus 25%; weight gain 4.5 Kg.; no palpable thyroid; "S": 1, 1a.
22. E.S.	40	F	Toxic diffuse 1942—Subtotal thyroidectomy	BMR plus 54% before operation; weight stationary; thyroid not palpable. <i>Persistent.</i> "S": 1, 1a, 2, 3, 4, 6, 8, 9.	Menopause	Lugol's solution without relief.	BMR plus 11%; bilateral thyroid enlargement; "S": 1, 1a, 2, 3, 4, 6, 8, 9.	Propylthiouracil 0.150 Gm. daily for 23 months; estrogens; still under treatment.	BMR minus 11%; no palpable thyroid; weight gain 3 Kg.; "S": 1, 1a, 6, 8.
23. J.M.	42	M	Toxic diffuse 1929—Subtotal thyroidectomy	BMR unknown; no weight change; thyroid not palpable. <i>Persistent.</i> "S": 1, 1a, 2, 3, 4, 6, 8. Frequent urticaria.	None	Lugol's solution indurable because of iodide rash.	BMR plus 30%; palpable rt. lobe and isthmus; weight stationary; "S": 1, 1a, 2, 3, 4, 6.	Thiouracil 0.4 Gm. daily for 9 months; propylthiouracil 0.05 Gm. daily for 1 month; no treatment for 24 months.	BMR minus 5%; no weight change; thyroid barely palpable; "S": 1, 1a, 6.
24. M.M.	64	F	Toxic diffuse 1923—Subtotal thyroidectomy	BMR unknown; weight loss unknown; thyroid not enlarged. <i>Recurrent</i> after 23 years. "S": 1a, 2, 3, 4, 6, 12.	Amputation of rt. leg for diabetic gangrene.	Diabetes difficult to control with diet and insulin.	BMR plus 14%; palpable substernal bilateral thyroid enlargement; weight loss unknown; diabetes hard to control; "S": 1a, 2, 3, 4, 6, 12.	Propylthiouracil 0.150 Gm. daily for 15 months; no treatment for 16 months.	BMR minus 8%; weight gain 3 Kg.; thyroid barely palpable; diabetes easily controlled; "S": 1a.
25. F.M.	39	F	Toxic diffuse 1926—Subtotal thyroidectomy	BMR unknown; weight loss unknown. <i>Persistent.</i> Thyroid enlarged. "S": 1, 1a, 2, 3, 4, 6, 9.	Psychic trauma (husband in military service.)	Sedatives, with temporary improvement.	BMR plus 44%; bilateral thyroid enlargement; weight loss 19 lbs.; cholestol 250 mg.; "S": 1, 1a, 2, 3, 4, 6, 9.	Thiouracil 0.4 Gm. daily for 9 months; then 0.2 Gm. daily for 3 months; then 0.1 Gm. daily for 12 months; propylthiouracil 0.100 Gm. daily for 15 months; no medication for 21 months.	BMR minus 18%; weight gain 9 Kg.; thyroid barely palpable; "S": 1a.

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or pre-clinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for \$1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to \$1,800.

* * * * *

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950*.

The 1950 Meeting of the American Goiter Association

The next meeting of the American Goiter Association will be held at the Hotel Shamrock, Houston, Texas, March 9, 10, and 11, 1950. It is recommended that all physicians wishing to attend make their hotel reservations early.

Award of the American Goiter Association

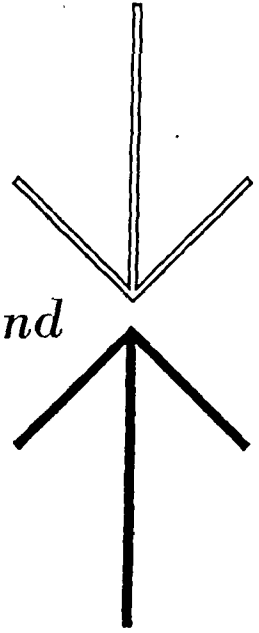
VAN METER PRIZE

THE AMERICAN GOITER ASSOCIATION again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Houston, Texas, March 9, 10 and 11, 1950, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed three thousand words in length, and must be presented in English. A typewritten double spaced copy *in duplicate* should be sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East St. Vrain Street, Colorado Springs, Colorado, not later than *January 15, 1950*. The Committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

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during the winter months. Similar thyroid enlargement was induced in rabbits fed on turnip. Surprisingly enough, however, the New Zealanders were unable to detect any appreciable activity in the native cabbage.

A few years later these same investigators made another important contribution when they discovered that the Brassica seed-goiters would not develop in the absence of an intact pituitary and that the administration of physiologic amounts of thyroid or thyroxine would also inhibit their development. The administration of iodine, however, although lessening the thyroid response to Brassica seed feeding by about one third, would not prevent the goitrogenesis. The mechanism of action therefore was concluded to be an inhibition of thyroxine synthesis (8, 9, 10, 11, 12). At the same time identical conclusions were reached concerning the mechanism of action of the antithyroid drugs (13, 14).

One in particular among many unanswered questions remained to stimulate those interested in the field. What was the nature of the active compound contained in these foods? Twenty years ago Marine had made investigations into this problem and had found that the active principle of cabbage was ether soluble, insoluble in water, volatile, and that the activity of the food could be increased by steaming or boiling. Since the mustard oils are the most characteristic compounds of this family and since they are both ether soluble and volatile, it was only natural that he should have first turned to these isothiocyanates. However, several different mustard oils had no effect when tested in rabbits.

Cyanides were also reported to be common constituents of the cabbage family, so these compounds were next tried. All cyanides tested were found to produce thyroid hypertrophy in the rabbits, but particularly methyl cyanide (acetonitrile), the simplest and least toxic. Marine (15) then postulated that the active material in cabbage was some sort of cyanide and that it produced thyroid enlargement by interfering with oxidation mechanisms in the tissues, thereby in some manner increasing the need for thyroid hormone. Several investigators since then, however, have been unable to confirm the goitrogenicity of cyanide, and at the present time the general consensus is that it is by no means proved that *any* cyanide can be incriminated as a naturally occurring goitrogen.

About this time, another great step forward was taken when Richter and Clisby (1941) in Baltimore reported that a substance they were testing as a taste discriminator, phenylthiourea, would produce thyroid enlargement in their rats (16). The following year Kennedy of the New Zealand group published a short report (17) suggesting that allylthiourea might be the active principle of cabbage and showing that it, too, was goitrogenic in rats. The reasoning behind Kennedy's suggestion was never made quite clear, but probably he put together the facts that allyl mustard oil was common

Although this theory has been demonstrated to be correct by innumerable investigators in innumerable experiments, and the rising use of iodized salt has been accompanied by a concomitant decrease in the incidence of goiter, the undeniable fact remains that not all nontoxic thyroid enlargement can be explained by postulating iodine-lack. It is not at all uncommon to find living in the vicinity of Boston, within a few miles of the ocean, many goitrous individuals who have been in this locality all their lives and who are accustomed to consuming sea food at least once a week. Surely the iodine ingestion in such cases must be well above the 100-200 micrograms estimated to be the daily requirement for man.

With the discovery that certain chemical compounds, the antithyroid drugs, would inhibit the formation of thyroxine, an entirely novel conception of human goitrogenesis was made possible. If such compounds were in some unexplained manner made available to the human organism during his daily routine, the pathogenesis of some heretofore inexplicable cases of thyroid enlargement might now be understood. All that was necessary was the demonstration of a naturally occurring goitrogen in substances that would ensure its access to the thyroid gland. Of course, the most obvious source of such a goitrogen would be in the food or water, and water could be excluded in most modern communities, since municipal water supplies serve the entire population.

The idea that certain foods may cause thyroid enlargement is not new. Many primitive peoples believe that this is the cause of their "swelled necks." No really good experimental evidence in support of this hypothesis was forthcoming, however, until the work of Chesney, Clawson, and Webster in 1928 (1, 2, 3), who discovered that rabbits being maintained in their laboratory were developing large goiters although their necks were perfectly normal upon purchase. After tracing down other possible factors, these investigators decided that the daily cabbage ration was responsible (4).

Marine and his collaborators in New York immediately confirmed and extended the original observations (5, 6), finding that several other Brassicae (the genus to which cabbage belongs) would produce thyroid hypertrophy when fed to rabbits, among them cauliflower and Brussels sprouts. Scientific workers from all over the world added further confirmation to this discovery and several new vegetables were added to the list. A few reports, of course, were made of failure to reduplicate Chesney's results.

No really new contribution was made to the field, however, until 1936, when Hercus and Purves (7) in New Zealand discovered that the seeds of certain of the Brassicae, notably rape seed, mustard seed, and cabbage seed, were effective goitrogens when fed to rats. They also reported an epidemic of goiter occurring among sheep which were fed a diet of turnip

serum iodide of these patients was approximately 0.5 micrograms per cent and their daily urinary excretion of iodide between 150 and 250 micrograms per diem. The unit of radioactivity used in Table 1 is the older Oak Ridge curie, which was 1.75 times the present Oak Ridge curie.

Precipitation of Serum Reinforced with 3, 5 Diiodotyrosine

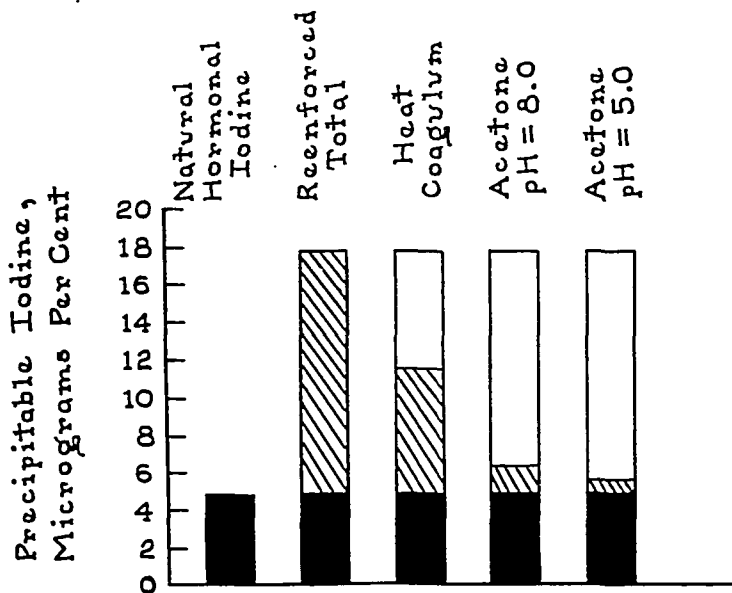


FIG. 3. When serum containing diiodotyrosine is precipitated by heat-coagulation a spuriously high value for protein-precipitable iodine is obtained. This error can be obviated largely by precipitating with acid-acetone.

The pulmonary clearance suggests that nearly 0.3 per cent of the body's reserve of iodide was exhaled per hour. Therefore, in the course of three or four weeks over 1 per cent of an assimilated dose might be lost by expiration, on the assumption that the loss by sweat was negligible. In extremely hot weather doubtless less would be exhaled proportionately, because the relative loss of water through the lungs would be less. This relation of iodide in expired water vapor to the iodide reserve in body water may afford a useful gauge of dosage during therapy with radioiodide.

The results just cited hold only when test or tracer doses are employed. When appreciable amounts of carrier iodide were also given, the loss of radioactive iodide by the urine was greater and less was trapped in the thyroid.

Organic iodine in human serum. When iodide is added to human serum in excess, as shown by Bassett, Coons and Salter (11), the heat-coagulated

TABLE 1. HUMAN PULMONARY CLEARANCE OF I^{131}

Patient	Days after oral dose	Radioactivity of 1 ml. serum		Expired air (counts per minute)*	Daily urinary excretion		
		Per cent of dose	curies $\times 10^{-3}$		I^{131} (per cent of dose)	I^{127} (μ g.)	Urine volume (ml.)
Ja. N. H. H. #C15816 <i>Test dose</i> (150 μ c.) <i>Therapeutic dose</i> (45.8 mc.) Male, 63 yrs. 83.6 Kg. B.M.R. = -45 per cent Thyroid carcinoma, metastatic in manubrium sterni, treated with I^{131} . Serum "hormonal" iodine = 0.9 μ g. % Serum iodide = 1.5 μ g. %	0.0						
	1.5	6.9 $\times 10^{-3}$	13	6,700			
	2.5			2,300			
	3.8	1.7 $\times 10^{-3}$	3.1	950			
	0.0						
	1.0	82 $\times 10^{-5}$	423	Off scale	35 (18 hrs.)	41	1,590
	1.75	35 $\times 10^{-5}$	159	Off scale	36	95	3,820
	3.75	30 $\times 10^{-5}$	138	Off scale	7.7	225	3,160
	6.0			10,815	2.6	170	1,890
	6.8	5.6 $\times 10^{-5}$	29	10,065			
	10.75	3.2 $\times 10^{-5}$	15	3,710			
Li. N. H. H. #49683 <i>Test dose</i> (140 μ c.) Male, 60 yrs. 60.1 Kg. Anaplastic cervical carcinoma Serum "hormonal" iodine = 4.1 μ g. % Serum iodide = 1.6 μ g. %	0.0						
	1.3			210	48	89	810
	1.8	2.1 $\times 10^{-3}$	4.0	—	2.6	130	1,120
Be. N. H. H. #B8364 <i>Test dose</i> (150 μ c.) <i>Therapeutic dose</i> (45.8 mc.) Female, 30 yrs. 53.2 Kg. B.M.R. = -30 per cent Recurrent thyroid carcinoma, treated with I^{131} Serum "hormonal" iodine = 2.1 μ g. % Serum iodide = 5.2 μ g. %	0.0						
	3.9	0.44 $\times 10^{-3}$	0.94	62	—		
	0.0						
	0.8	17 $\times 10^{-5}$	80	Off scale	50	205	3,610
	1.8	12 $\times 10^{-5}$	56	Off scale	19.8	206	3,440
				23,000 —	2.5	138	3,380
	3.8	8.6 $\times 10^{-5}$	40	10,300	0.75	55	2,980
	4.8			6,000			
	5.8	5.0 $\times 10^{-5}$	23	4,400			
	7.0			4,050			
	17.0	0.63 $\times 10^{-5}$	2.9	410			
Ma. N. H. H. #C27671 <i>Test dose</i> (147 μ c.) Female, 29 yrs. 54.1 Kg. B.M.R. = +1 per cent Thyroid carcinoma with lateral node metastasis Serum "hormonal" iodine = 4.6 μ g. % Serum iodide = 1.9 μ g. %	0.0						
	1.0	13 $\times 10^{-3}$	18.7	2,500			

* Readings taken with Tracerlab, Inc. (Boston) Model SU-3A laboratory monitor equipped with Geiger tube TGC-1 through probe window 2.8 cm. in diameter.

tracer radioiodide for several patients at both the "test-dose" and the "therapeutic-dose" levels. The pulmonary clearance is given in detail for patients Ja and Be, who suffered from thyroid cancer. Both patients were ambulatory and active, although the first was being treated for a metastasis in the manubrium sterni and the latter for the thrice-recurrent growth of a thyroid adenopapilloma, still confined to the cervical region. The

Metabolic experiments (rats)

Methods

The metabolic fates of iodide, diiodotyrosine, phenyl diiodohydroxy phenyl propionic acid ("Priodax") and phenyl diiodohydroxy cinnamic acid (called P-76) were studied in adult male Wistar rats, weighing nearly 300 grams each. Both stable and radioactive forms of these substances were used. The material was injected intraperitoneally in physiologic saline solution. The dosage was adjusted in each case to yield 500 micrograms of total iodine per 100 grams of rat. The animals were fasted overnight before the injection. They were sacrificed at increasing intervals after the injection, so that a composite curve of iodine concentration could be attained. In order to conserve the brain, each animal was rapidly reduced to the beginning of Stage II anesthesia with diethyl ether and then exsanguinated by carotid bleeding. Care was taken not to injure the thyroid gland. Various organs were then removed as rapidly as possible, weighed and chilled in an ice box at 4° C. The blood was treated similarly.

From each organ approximately 900 milligrams was taken and weighed. This sample was finely ground with a miniature conical mortar and pestle in order to homogenize it. Distilled water, made alkaline with ammonia to a concentration of 0.015 normal, was used to dissolve the protoplasmic constituents. The final homogenate had a volume of about 6 ml., which was collected in a 15 ml. conical centrifuge tube. With 1 N acetic acid the turbid solution or suspension was brought to the isoelectric point near pH 5.0, and the centrifuge tube was heated to about 95°C. in a boiling water bath. As the protein coagulated it was stirred intermittently to prevent the formation of large masses of coagulum. After standing ten minutes at 95°C., the tube was centrifuged and the supernatant liquor saved. The coagulum was washed once with 2 ml. of water and centrifuged again.

The combined supernatant liquors were divided. Half was used to measure radioactivity and half to measure stable iodine. In the latter instance, the iodine extractable with butyl alcohol was separated from the hydrophilic iodide. The heat-coagulum was dissolved in 1 ml. of warm 1 N sodium hydroxide.

The measurements of radioactivity were made according to the method described by Salter (16, 17) in which the unknown, X , is compared with $X + a$, where " a " is a known aliquot of the original radioactive material. This method obviates the need for ashing the sample and at the same time compensates for various geometric factors and errors due to internal absorption or adsorption. Stable I^{127} was measured by the procedure described in detail by Salter and Johnston (5).

As an alternate method to heat coagulation near pH 5.0, certain homogenates were also precipitated with cold acetone at pH 5.0, as described by Salter and Johnston (5).

Because the measurements of radioactivity were detailed and laborious, comparative measurements were made at a time when the distribution of iodine had become somewhat stable. This appeared to be most illuminating three hours after injection, but probably it would be interesting to carry out the detailed analysis at longer intervals. At any rate, the detailed